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PROVISIONAL

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Inventor(s): CARTER, Darrick

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☐ Combined Declaration & Power of Attorney  
☐ Assignment ☐ Small Entity Decl.

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Microfiche Appendix: \_\_\_\_\_

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PROVISIONAL APPLICATION

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Transmitted herewith for filing is a provisional patent application under CFR 1.53(c) of:

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (City/State/Country)
CARTER	Darrick		

Title: IMMUNE MEDIATORS AND RELATED METHODS

Enclosed are:

- ☒ 43 pages of the specification (including description and claims).  
☒ 1 page of Abstract.  
☐ The invention was made by or under a contract with the following agency of the United States Government:

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**PROVISIONAL PATENT APPLICATION**  
**IMMUNE MEDIATORS AND RELATED METHODS**

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10

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# IMMUNE MEDIATORS AND RELATED METHODS

## CROSS-REFERENCES TO RELATED APPLICATIONS

The present application is related to U.S. Serial No. 09/261811, filed  
5 March 3, 1999, which is a continuation-in-part of U.S. Serial No. 08/480,002, filed June  
7, 1995, now abandoned, U.S. Serial No 08/657,581 filed June 7, 1996, now abandoned  
and U.S. Serial No 09/184,692 November 2, 1998, all of which are incorporated herein by  
reference.

## BACKGROUND OF THE INVENTION

10 There is currently a great interest in developing pharmaceuticals based on  
the growing understanding of the structure and function of the major histocompatibility  
complex (MHC) antigens. These cell surface glycoproteins are known to play an  
important role in antigen presentation and in eliciting a variety of T cell responses to  
antigens. Identification of synthetic antigenic peptides, and demonstration that these  
15 peptides bind selectively to MHC molecules associated with disease and that stimulates T  
cells would help to implicate a particular peptide or peptide:MHC complex in  
susceptibility to an autoimmune disease. In particular, the development of soluble, fused  
MHC heterodimer:peptide complexes would be particularly useful in treatment of a  
number of diseases associated with antigen presentation by MHC molecules. The present  
20 invention fulfills such needs, and provides related advantages.

## SUMMARY OF THE INVENTION

The present invention provides recombinant DNA constructs that encode  
soluble, fused MHC heterodimers that may or may not be further linked to an antigenic  
25 peptide. Typically, the constructs comprise a first DNA segment encoding at least a  
portion of a first domain of a selected MHC molecule; a second DNA segment encoding  
at least a portion of a second domain of the selected MHC molecule; a first linker DNA  
segment and connecting in-frame the first and second DNA segments; wherein linkage of  
the first DNA segment to the second DNA segment by the first linker DNA segment  
30 results in a fused first DNA-first linker-second DNA polysegment. The constructs of the  
invention may also comprise a third DNA segment encoding an antigenic peptide capable



of associating with a peptide binding groove of the selected MHC molecule and a second linker DNA segment connecting in-frame the third DNA segment to the fused first DNA-first linker-second DNA polysegment. The linkage of the third DNA segment to the fused first DNA-first linker-second DNA polysegment by the second linker DNA segment results in a soluble, fused MHC heterodimer:peptide complex.

As shown below, the invention provides preferred constructs, comprising for example preferred linker sequences. The linkers and other sequences used in the constructs of the invention are particularly useful in producing complexes that fold properly and have enhanced therapeutic activity.

### DESCRIPTION OF THE SPECIFIC EMBODIMENTS

Prior to setting forth the invention, it may be helpful to an understanding thereof to provide definitions of certain terms to be used hereinafter:

Fused MHC heterodimer:peptide complex (also referred to as half-anergix): As used herein it refers to a fusion protein such as the fused, MHC heterodimer:peptide complex of the invention. Such fusion proteins will be indicated with a colon (:). MHC-peptide complexes which are not, fusion proteins, are native MHC containing protein or exogenously loaded MHC molecules are indicated with a dash (-).

A domain of a selected MHC molecule: A portion of an MHC domain which is sufficient to form, either alone, or in combination with another portion of an MHC domain, a peptide binding site which is capable of presenting an antigenic peptide in such a fashion that it is recognized by a T cell receptor. Such MHC domains would include the extracellular portion of the two polypeptide chains of either Class I or Class II MHC. This would include any or all of the domains of  $\alpha$  chain ( $\alpha 1$ ,  $\alpha 2$ , or  $\alpha 3$ ) and  $\beta 2$ -microglobulin subunit of Class I MHC. For example, Class I MHC domains would include any combination of the three  $\alpha$  chain domains either independent of the others,  $\alpha 1$ ,  $\alpha 2$ , or  $\alpha 3$ , in tandem,  $\alpha 1\alpha 2$ ,  $\alpha 2\alpha 3$ ,  $\alpha 1\alpha 3$ , and/or the  $\beta 2$  domain. Also included are the  $\alpha$  chain ( $\alpha 1$ ,  $\alpha 2$ ) and  $\beta$  chain ( $\beta 1$ ,  $\beta 2$ ) of Class II MHC. This would include  $\alpha 1$  or  $\alpha 2$  independent of the other, or  $\alpha 1$  and  $\alpha 2$  in tandem ( $\alpha 1\alpha 2$ ). It would also include  $\beta 1$  or  $\beta 2$  independent of the other, or  $\beta 1$  and  $\beta 2$  in tandem ( $\beta 1\beta 2$ ).

Linker DNA segment: A segment of DNA (typically encoding about 5 to about 25 amino acids), prototypically repeating glycine residues with interspersed serine residues which forms a flexible link between two DNA segments. This flexible link

allows the two DNA segments to attain a proper configuration, such as an MHC peptide binding groove, or allows a peptide to properly bind into such a groove.

Antigenic peptide: A peptide which contains an epitope recognized by immune cells, particularly T cells, and is capable of stimulating an MHC-mediated immune response.

The complexes of the invention can be made according to standard recombinant techniques described, for example in the above referenced related applications, WO 96/40944 and in US Patent No. 5, 869,270. These patent documents also provide description of how to use the complexes to treat diseases, for example, autoimmune diseases.

The following examples are offered by way of illustration, not by way of limitation.

## EXAMPLES

### EXAMPLE 1

Construction of DNA sequences encoding human soluble, fused MHC heterodimer:peptide complexes, HLA-DR4 molecules(CO563 or CO564).

DNA constructs encoding gp39  $\beta 1/\alpha 1$  human molecules, were prepared from a cDNA encoding the gp39 peptide fused to the  $\beta 1/\alpha 1$  domains of HLA-DR4 according to standard techniques.

For the production of "empty"  $\beta 1/\alpha 1$  DR4 molecules, a cDNA encoding the fused  $\beta 1/\alpha 1$  domains of HLA-DR4 was prepared using cloned  $\alpha$  and  $\beta$  chains from DR4.

Amino acid sequence of the gp39- $\beta 1/\alpha 1$  HLA-DR4 human half anergic molecule (linkers are shown in bold)

MGDTGRSFTLASSETGVGASGGGGSGGGGDTRPRFLEQVKHECH  
FFNGTERVRFLDRYFYHQEEYVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQ  
KRAAVDTYCRHNYGVGESFTVQRRGGIKEEHVIIQAEFYLNPDQSGEFMFDFDG  
DEIFHVDMAKKETVWRLEEFGRFASFEAQGALANLAVDKANLEIMTKRSNYTPIT  
N\*

aa1 - aa4 : leader sequence

aa5 - aa18 : gp39 peptide

aa19 - aa28 : linker

aa29 - aa122 : HLA-DR4  $\beta$ 1 domain

aa123 - aa124 : linker

aa125 - aa 208: HLA-DR4  $\alpha$ 1 domain

5           Amino acid sequence of the “ empty ”  $\beta$ 1/ $\alpha$ 1 HLA-DR4 human half  
anergix molecule

MGDTRPRFLEQVKHECHFFNGTERVRFLLDRYFYHQEEYVRFDSDV  
GEYRAVTELGRPDAEYWNSQRDLLEQKRAAVDTYCRHNYGVGESFTVQRRGGI  
KEEHVIIQAEFYLNPDQSGEFMFDFDGD EIFHVDMAKKETVWRLEEFGRFASFEA  
10 QGALANIAVDKANLEIMTKRSNYTPITN\*

aa 1 - aa95: HLA-DR4  $\beta$ 1 domain

aa96- aa97 : linker

aa98- aa181 : HLA-DR4  $\alpha$ 1 domain

### 15           3- Production and purification of half anergix molecules.

For the production of recombinant proteins, the bacteria (pLysS) were grown in LB (containing ampicilline (50  $\mu$ g / ml) and chloramphenicol (5  $\mu$ g / ml)) at 37°C until OD600 = 0.5. IPTG was added at the final concentration of 0.5 mM final and the bacteria were further incubated for 3 hours at 37°C with shaking. The bacteria were  
20 centrifuged at 4°C, 4K for 20 min and the pellet was frozen at -80°C. The following day, the pellet was resuspended in 40 ml of lysis buffer (50 mM Tris-HCl pH8, 50 mM NaCl, 2 mM EDTA, 1 protease inhibitor cocktail tablet, 1% Triton X100 and 1% deoxycholate), and incubated for 1 hour at 4°C under continuous agitation with a magnetic stirrer. The sample was then homogenized using a French Press with a 1000 psi setting, and  
25 centrifuged at 4°C, 9000g for 20 min. The pellet was then resuspended in 30 ml of lysis buffer without Triton and deoxycholate and centrifuged at 4°C, 9000 g for 20 min. The new pellet was resuspended in 10 ml of 20 mM ethanolamine/6 M urea pH10, and eventually frozen at -80°C. The recombinant protein was then purified by FPLC ion-exchange chromatography using Source 30Q anion-exchange media in an XK26/20  
30 column using a step gradient going from 1 mM to 1 M NaCl in 20 mM ethanolamine/6M urea pH10. Fractions were analysed by SDS/PAGE and those corresponding to the proteins of interest are pooled and dialysed against PBS 1X.

## EXAMPLE 2

### Production of Additional Constructs

Additional constructs with different composition and length of the 2<sup>nd</sup> linker (between  $\beta 1$  and  $\alpha 1$ ) were engineered by using standard techniques using CO567 as the template. Specifically, PCR primers were designed to replace the old sequence in CO567 with the new sequence. For example to make CO581, the primers were designed with the following sequences (note these primers were phosphorylated at 5').

Primer 1: 5'

pCACCAGGAGGAGAGCCGCCACGCCGGTCTCGCTGG

Primer 2: 5' pGACCACCTGGATCTGGGGACACCCGACCACGTTTC

PCR reaction (100  $\mu$ l) was made of the following components: 2  $\mu$ l CO567 (80 ng) as template, 2  $\mu$ l each of primer 1 and primer 2 (10  $\mu$ M), 2  $\mu$ l of dNTP mix (20 mM each), 10  $\mu$ l of 10X pfu buffer, and 80  $\mu$ l of sterile water. After all the components were mixed, 2  $\mu$ l of Turbo pfu (5U total) was added, mixed and put on PCR machine. The PCR cycles has a pre-denaturation at 95C for 30 sec, then 10 cycles of 95C for 30 C, 60C r 1 min, and 72C for 7 min. Then another 22 cycles of 95C for 30C, 65C for 1 min and 72C for 7 min, followed by a final 10 min at 72C.

The PCR mixture was digested with 2  $\mu$ l of dpn I (10U) for 2h at 37C. Then the PCR product at ~6 kb was purified from agarose gel after electrophoresis. The purified PCR product was ligated by T4 DNA ligase for 1 h at room temperature then used to transform into NovaBlue (Novagen)competent cells by standard protocol. Cells were plated on LB (+Carb) and grow overnight at 37C.

Next day, about a dozen single colony from the transformation were randomly picked for overnight culture in 5 ml LB (+Carb) at 37 C. Plasmids from these culture were purified with Wizard Miniprep kit, and analyzed by Xho I digestion. A few plasmids that passed the Xho I digestion were further confirmed by DNA sequencing.

To express the recombinant proteins, a clone with confirmed DNA sequence was used to transform BL21(DE3)CodonPlus-RIL (Stratagene) by standard transformation protocol and plated on LB (Carb+Cam) plates overnight at 37C. Next

morning, a single colony was picked to inoculate 100 ml LB (+Carb +Cam) and the culture was grown till OD reach between 0.8-1.0, and stored overnight at 4C. Next day, the culture was pellet down and used to inoculate into 2xYT (+Carb+Cam) at ratio of 25 ml culture per liter new media. These large cultures were grown at 37C till OD=0.5-0.6, and IPTG was added to induce recombinant protein at 37C for 3 h. The induced cultures were pellet and stored at -80C till purification.

Table 1 provides a listing of various constructs made according to the invention.

Construct	peptide	upstream linker	downstream linker
C0523	yes	GGGG	GG
C0543	none	none	GG
C0563	yes	ASGGSGGG	GG
C0567	yes	ASGGSGGG	TSGGGSGGGSSS
C0580	yes	ASGGSGGG	GSPGGSGGGPGS
C0581	yes	ASGGSGGG	GSPPGGPPGS
C0582	yes	ASGGSGGG	GSPGGGGPGS
C0583	yes	ASGGSGGG	TSGGGGS
C0584	yes	ASGGSGGG	SGSGGS
C0585	yes	ASGGSGGG	FDAPSPLP
C0586	none	none	TSGGGSGGGSSS
C0587	none	none	GSPGGSGGGPGS
C0588	none	none	GSPPGGPPGS
C0589	none	none	GSPGGGGPGS
C0590	none	none	TSGGGGS
C0591	none	none	SGSGGS
C0592	none	none	FDAPSPLP
C0593	yes	ASGGSGGG	VYPEVTV
C0594	none	none	VYPEVTV
C0595	yes	ASGGSGGG	GGG
C0596	yes	ASGGSGGG	GGGS
C0597	yes	ASGGSGGG	GGSGG

Table 1

### EXAMPLE 3

The need for novel linker sequences was realized after inspection of circular dichroism spectra of purified, refolded ½ Anergix constructs. Inspection of the atomic structures of the various murine and human MHC class II molecules as  
5 determined by X-ray crystallography indicated that these molecules have a high degree of structural similarity. The circular dichroism results were consistent with two folded molecules of clearly different secondary structure. Careful inspection of the structures reveals that the human MHC has a longer distance between chains than the equivalent murine molecule. This led to proposing longer linkers between the chains which would  
10 contain flexible residues (e.g. alanine or glycine) and polar residues (e.g. serine and threonine). These constructs make up the first class of linkers. To inhibit the continuation of secondary structure across the linker, prolines were added to bracket the linkers. These prolines are known to inhibit the formation of alpha helices and beta sheets. These linkers make up the second class of linkers disclosed here.

15 Next, flexible regions present in the human MHC and in the murine MHC could be used to make a linker by extending the region of interest and ligating the ends together. These are the third class of linkers. Finally, a combination of these types of linkers could also be used. These are the fourth class of linkers.

### EXAMPLE 4

20 As it was known that the murine ½ Anergix construct is functional, another linker has been suggested based on a fusion of the two molecules. This fusion would incorporate residues from the functional murine ½ Anergix as a linker and the alpha and beta chains of the human molecule would be used to give the desired function.

25 The protein sequence of the molecule to be expressed is below:

MGDTGRSFTLASSETGVGASGGGGSGGGGDTRPRFLEQVKHECHFFNGTERVRF  
LDRYFYHQEEYVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQKRAAVDTYC  
RHNYGVGESFTVLRRLGGEDDEADHHVIIQAEFYLNPDQSGEFMFDFDGD EIFH  
30 VDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPITN\*

The linker residues from the mouse construct are in bold.

### EXAMPLE 5

Further MHC Class II hybrid molecules may be design by fusing other portions of the alpha chain and the beta chain together using linkers as described

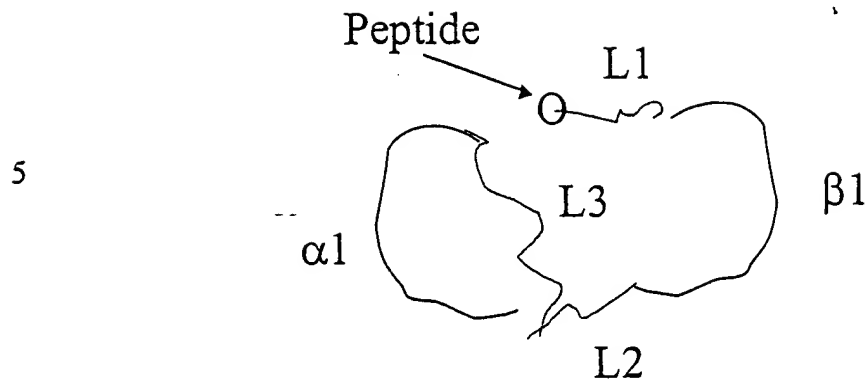
elsewhere. A properly folded molecule may be obtained by putting appropriate linkers between portions of the human MHC class II which are proximal to each other as determined by visual inspection of the atomic coordinates of residues of the native MHC available in the publicly accessible protein structure database. These structures would  
5 predict possible fusion proteins which covalently attach any part of the beta chain between residues 82 to 123 or between residues 148 to 164 to portions of the alpha chain such as the N-terminal residues, residues 79 to 84, or 92 to 106. Which combination of linker / domain from alpha chain / domain from beta chain is preferable remains to be determined by producing combinatorial fusions between these areas. The numbering  
10 system of residues in this example corresponds to those found in the coordinates of the structure described in: DESSEN, *et al. Immunity* 7:473 (1997). Other, homologous residues could be used to create equivalent constructs for genotypic and allelic variants of these molecules e.g. equivalent residues in DR2 or such. DNAs for such hybrids would be prepared and expressed in a recombinant expression system by someone skilled in the art  
15 and could be assayed for structure and function in appropriate assays.

#### EXAMPLE 6

Use of CD4 binding site(s) of MHC class II molecules as linkers for the production of bioactive recombinant MHC peptide truncated molecules

20 HLA class II molecules present antigenic peptides to the T cell receptor of the CD4+ T lymphocytes and interact with CD4 during the antigen recognition process. Native MHC class II-peptide complexes have been shown to bind to MHC class II restricted and antigen specific TCRs on a particular T cell and induce T cell nonresponsiveness. It is proposed that the CD4 binding site is important in the docking of  
25 MHC class II-Peptide complex with the TCR and induce nonresponsiveness. Since the binding of CD4 to MHC class II-peptide is important in antigen presentation and/or induction of T cell nonresponsiveness, we propose that recombinant MHC class II-peptide molecules (truncated or whole) containing CD4 binding site will be biologically active. We further propose that a polypeptide fragment from MHC class II which binds  
30 the CD4 if used as a linker in preparation of MHC class II-peptide truncated molecules the resulting recombinant molecules will be biologically active.





10

The above Figure shows the concept of different linkers. Peptide is attached to a linker 1 (L1) which is attached to N-terminus of  $\beta 1$  domain that is linked to L2. L2 is linked to L3, which in turn is linked to N-terminus of  $\alpha 1$  chain of MHC class II. Here L2  
 15 represents the human CD4 binding sequences. It should be noted that L2 could also be directly linked to N-terminus of  $\alpha 1$  domain by completely deleting L3.

Specific examples of L1 and L3 are given in examples.

20 The sequences of L2 are given below. These sequences are applicable to most of the DR-Peptide molecules.

1. RNGQEEKAGVVSTGLI
2. RNGQETKAGVVSTGLI
- 25 3. YNQQEEKAGGVSTGLI
4. FRNGQEEKAGVVSTGLI
5. FRNGQETKAGVVSTGLI
6. FYNQQEEKAGGVSTGLI
7. LNGQEEKAGMVSTGLI

30

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the

invention. Accordingly, the invention is not limited except as by the appended claims.  
All patents, patent applications, and publications are incorporated herein by reference.

# SEQUENCE LISTING

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 ttcgacagcg acgtggggga gtaccgggcg gtgacggagc tggggcggcc tgatgccgag 180  
 tactggaaca gccagaagga cctcctggag cagaagcggg ccgcggtgga cacctactgc 240  
 agacacaact acgggggttg tgagagcttc acagtgcagc ggcgaagtgg cggtagtggc 300  
 ggtagtatca aagaagaaca tgtgatcctc caggccgagt tctatctgaa tctgaccaa 360  
 20 tcaggcgagt ttatgttga ctttgatggt gatgagatt tccatgtgga tatggcaaag 420  
 aaggagacgg tctggcggct tgaagaattt ggacgattt ccagcttga ggctcaaggt 480  
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 tatactccga tcaccaatta a 561

25 <210> 8 CO590  
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 <212> DNA  
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 tactggaaca gccagaagga cctcctggag cagaagcggg ccgcggtgga cacctactgc 240  
 35 agacacaact acgggggttg tgagagcttc acagtgcagc ggcgaactag tgggtggcgg 300  
 ggcagcatca aagaagaaca tgtgatcctc caggccgagt tctatctgaa tctgaccaa 360  
 tcaggcgagt ttatgttga ctttgatggt gatgagatt tccatgtgga tatggcaaag 420  
 aaggagacgg tctggcggct tgaagaattt ggacgattt ccagcttga ggctcaaggt 480  
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 45 <213> Homo sapiens

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10 <211> 570

<212> DNA

<213> Homo sapiens

<400> 10

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 ttgacagcg acgtggggga gtaccgggcg gtgacggagc tggggcggcc tgatgccgag 180  
 tactggaaca gccagaagga cctcctggag cagaagcggg ccgcggtgga cacctactgc 240  
 agacacaact acgggggttgg tgagagcttc acagtgcagc ggcgaggctc tcctcctggt 300  
 20 ggaccacctg gatctatcaa agaagaacat gtgatcatcc aggccgagtt ctatctgaat 360  
 cctgaccaat caggcgagtt tatgtttgac ttgatgtg atgagattt ccatgtggat 420  
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25 <210> 11 CO587

<211> 582

<212> DNA

<213> Homo sapiens

30 <400> 11

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 ttgacagcg acgtggggga gtaccgggcg gtgacggagc tggggcggcc tgatgccgag 180  
 35 tactggaaca gccagaagga cctcctggag cagaagcggg ccgcggtgga cacctactgc 240  
 agacacaact acgggggttgg tgagagcttc acagtgcagc ggcgaggag tccaggcggg 300  
 ggcgggagcg gcggtgtcc tgggagtatc aaagaagaac atgtgatcat ccaggccgag 360  
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 ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgatt 480  
 40 gccagcttg aggtcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 540  
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<210> 12 CO586

<211> 582

45 <212> DNA

<213> Homo sapiens

<400> 12

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 ttccatgtgg atatggcaaa gaaggagacg gtcctggcggc ttgaagaatt tggacgatt 480  
 gccagcttgg aggcctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 540  
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10 <210> 13 CO602

<211> 186

<212> PRT

<213> Homo sapiens

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His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
                   20                  25                  30

20

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
                   35                  40                  45

25 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
                   50                  55                  60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
                   65                  70                  75                  80

30

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Leu Arg Arg Leu  
                   85                  90                  95

Gly Gly Glu Asp Asp Glu Ala Asp His His Val Ile Ile Gln Ala Glu  
                   100                  105                  110

35

Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp  
                   115                  120                  125

40 Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp  
                   130                  135                  140

Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala  
                   145                  150                  155                  160

45

Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys  
                   165                  170                  175

Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
                   180                  185

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<210> 14 CO601  
 <211> 186  
 5 <212> PRT  
 <213> Homo sapiens  
  
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           His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
                   20                  25                  30  
  
 15 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
                   35                  40                  45  
  
           Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
                   50                  55                  60  
 20 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
                   65                  70                  75                  80  
  
           Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly  
 25                  85                  90                  95  
  
           Gly Gly Ser Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu  
                   100                  105                  110  
  
 30 Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp  
                   115                  120                  125  
  
           Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp  
                   130                  135                  140  
 35 Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala  
                   145                  150                  155                  160  
  
           Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys  
 40                  165                  170                  175  
  
           Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
                   180                  185  
  
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 <210> 15 CO600  
 <211> 185  
 <212> PRT  
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<400> 15

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
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5 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
35 40 45

10 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
50 55 60

15 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly  
85 90 95

20 Gly Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe  
100 105 110

Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly  
115 120 125

25 Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg  
130 135 140

30 Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu  
145 150 155 160

Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg  
165 170 175

35 Ser Asn Tyr Thr Pro Ile Thr Asn  
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<210> 16 CO599

40 <211> 184

<212> PRT

<213> Homo sapiens

<400> 16

45 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
20 25 30

50

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45

5 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80

10 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly  
 85 90 95

Gly Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr  
 100 105 110

15 Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp  
 115 120 125

Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu  
 20 130 135 140

Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala  
 145 150 155 160

25 Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser  
 165 170 175

Asn Tyr Thr Pro Ile Thr Asn  
 180

30

<210> 17 CO594  
 <211> 187  
 <212> PRT  
 35 <213> Homo sapiens

<400> 17  
 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
 5 10 15

40 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
 20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 45 35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60

50 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys

	65	70	75	80
	Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Val			
	85	90	95	
5	Tyr Pro Glu Val Thr Val Ile Lys Glu Glu His Val Ile Ile Gln Ala			
	100	105	110	
	Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe			
10	115	120	125	
	Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val			
	130	135	140	
15	Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly			
	145	150	155	160
	Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr			
	165	170	175	
20	Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn			
	180	185		
25	<210> 18 CO592			
	<211> 188			
	<212> PRT			
	<213> Homo sapiens			
30	<400> 18			
	Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys			
	5	10	15	
	His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe			
35	20	25	30	
	Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr			
	35	40	45	
40	Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser			
	50	55	60	
	Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys			
	65	70	75	80
45	Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Phe			
	85	90	95	
	Asp Ala Pro Ser Pro Leu Pro Ile Lys Glu Glu His Val Ile Ile Gln			
50	100	105	110	

Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp  
 115 120 125

5 Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr  
 130 135 140

Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln  
 145 150 155 160

10 Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met  
 165 170 175

Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
 15 180 185

<210> 19 CO591  
 <211> 187  
 20 <212> PRT  
 <213> Homo sapiens

<400> 19  
 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
 25 5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
 20 25 30

30 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60

35 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Ser  
 40 85 90 95

Gly Gly Ser Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala  
 100 105 110

45 Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe  
 115 120 125

Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val  
 130 135 140

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Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly  
 145                      150                      155                      160

5      Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr  
                     165                      170                      175

Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
                     180                      185

10      <210> 20 CO590  
          <211> 187  
          <212> PRT  
          <213> Homo sapiens

15      <400> 20  
 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
                     5                      10                      15

20      His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
                     20                      25                      30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
                     35                      40                      45

25      Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
                     50                      55                      60

30      Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
                     65                      70                      75                      80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr  
                     85                      90                      95

35      Ser Gly Gly Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala  
                     100                      105                      110

Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe  
                     115                      120                      125

40      Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val  
                     130                      135                      140

45      Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly  
                     145                      150                      155                      160

Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr  
                     165                      170                      175

50      Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn

180

185

&lt;210&gt; 21 CO589

5 &lt;211&gt; 190

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 21

10 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
                   5                  10                  15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
                   20                  25                  30

15 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
                   35                  40                  45

20 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
                   50                  55                  60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
                   65                  70                  75                  80

25 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly  
                   85                  90                  95

Ser Pro Gly Gly Gly Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile  
                   100                  105                  110

30 Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met  
                   115                  120                  125

35 Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys  
                   130                  135                  140

Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu  
                   145                  150                  155                  160

40 Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu  
                   165                  170                  175

Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
                   180                  185

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&lt;210&gt; 22 CO588

&lt;211&gt; 190

&lt;212&gt; PRT

50 &lt;213&gt; Homo sapiens

<400> 22

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
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5

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
20 25 30

10

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
50 55 60

15

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly  
85 90 95

20

Ser Pro Pro Gly Gly Pro Pro Gly Ser Ile Lys Glu Glu His Val Ile  
100 105 110

25

Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met  
115 120 125

Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys  
130 135 140

30

Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu  
145 150 155 160

Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu  
165 170 175

35

Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
180 185

40

<210> 23 CO587

<211> 194

<212> PRT

<213> Homo sapiens

45

<400> 23

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
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His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45

5 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80

10 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly  
 85 90 95

Ser Pro Gly Gly Gly Gly Ser Gly Gly Gly Pro Gly Ser Ile Lys Glu  
 15 100 105 110

Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser  
 115 120 125

20 Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp  
 130 135 140

Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe  
 145 150 155 160

25 Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys  
 165 170 175

Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr  
 30 180 185 190

Asn

35  
 <210> 24 CO586  
 <211> 194  
 <212> PRT  
 <213> Homo sapiens

40  
 <400> 24  
 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
 5 10 15

45 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
 20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45

50



Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60

5 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr  
 85 90 95

10 Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Ile Lys Glu  
 100 105 110

Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser  
 115 120 125

15 Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp  
 130 135 140

Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe  
 20 145 150 155 160

Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys  
 165 170 175

25 Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr  
 180 185 190

Asn

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<210> 25 CO598  
 <211> 639  
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35 <213> Homo sapiens

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 40 catgagtgtc atttctcaa cgggacggag cgggtgcggt tctggacag atactctat 180  
 caccaagagg agtacgtgcg ctgcgacgc gacgtggggg agtaccgggc ggtgacggag 240  
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 gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgtg 360  
 cgccgactcg gaggtgaaga tgacgaggca gatcaccatg tgatcatcca ggccgagttc 420  
 45 tatctgaatc ctgaccaatc aggcgagttt atgtttgact ttgatgtga tgagatttc 480  
 catgtggata tggcaaagaa ggagacggtc tggcggcttg aagaatttg acgattgcc 540  
 agctttgagg ctcaaggtgc attggccaac atagctgtgg acaaagcaa cctggaaatc 600  
 atgacaaagc gctccaacta tactccgatc accaattaa 639

50 <210> 26 CO597

<211> 639  
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<213> Homo sapiens

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catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcttgacag atacttctat 180  
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
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gccgcggttg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360  
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tatctgaatc ctgaccaatc aggcgagttt atgtttgact ttgatgtga tgagatttc 480  
catgtggata tggcaaagaa ggagacggtc tggcggcttg aagaatttg acgatttgcc 540  
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atgacaaagc gctccaacta tactccgac accaattaa 639

<210> 27 CO596

<211> 636

20 <212> DNA

<213> Homo sapiens

<400> 27

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catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcttgacag atacttctat 180  
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
gccgcggttg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360  
30 cggcgaggag gtggaggcag catcaaagaa gaacatgtga tcatccaggc cgagttctat 420  
ctgaatcctg accaatcagg cgagtttat tttgacttg atggtgatga gatttccat 480  
gtggatatgg caaagaagga gacggtctgg cggcttgaag aatttgacg atttgccagc 540  
tttagggctc aaggtgcatt ggccaacata gctgtggaca aagccaacct ggaaatcatg 600  
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<210> 28 CO595

<211> 633

<212> DNA

<213> Homo sapiens

40 <400> 28

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catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcttgacag atacttctat 180  
45 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
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gccgcggttg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360  
cggcgaggag gtggaggcat caaagaagaa catgtgatca tccaggccga gttctatctg 420  
aatcctgacc aatcaggcga gtttatgtt gactttgatg gtgatgagat ttccatgtg 480  
50 gatatggcaa agaaggagac ggtctggcgg ctgaagaat ttggacgatt tgccagcttt 540

gaggctcaag gtgcattggc caacatagct gtggacaaag ccaacctgga aatcatgaca 600  
aagcgtcca actatactcc gatcaccaat taa 633

<210> 29 CO593

5 <211> 642

<212> DNA

<213> Homo sapiens

<400> 29

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catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
15 gccgcggtgg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360  
cggcgagtct accctgaggt aactgtcatc aaagaagaac atgtgatcat ccaggccgag 420  
ttctatctga atcctgacca atcaggcgag ttatgttg actttgatgg tgatgagatt 480  
ttccatgtgg atatggcaa gaaggagacg gtcggcggc ttgaagaatt tggacgattt 540  
gccagcttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600  
20 atcatgacaa agcgtccaa ctatactcg atcaccaatt aa 642

<210> 30 CO585

<211> 645

<212> DNA

25 <213> Homo sapiens

<400> 30

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30 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
gccgcggtgg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360  
cggcgattcg acgcacctag cccactccca atcaaagaag aacatgtgat catccaggcc 420  
35 gagtctatc tgaatcctga ccaatcaggc gagtttatgt ttgacttga tggatgatgag 480  
attttccatg tggatatggc aaagaaggag acggtctggc ggcttgaaga atttgacga 540  
tttgccagct ttgaggctca aggtgcattg gccaacatag ctgtggacaa agccaacctg 600  
gaaatcatga caaagcgtc caactatact ccgatcacca attaa 645

40 <210> 31 CO584

<211> 642

<212> DNA

<213> Homo sapiens

45 <400> 31

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catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
50 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300

gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360  
 cggcgaagtg gcggtagtgg cggtagtatc aaagaagaac atgtgatcat ccaggccgag 420  
 ttctatctga atcctgacca atcaggcgag ttatgtttg actttgatgg tgatgagatt 480  
 ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 540  
 5 gccagctttg aggtcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600  
 atcatgacaa agcgtccaa ctatactccg atcaccaatt aa 642

<210> 32 CO583  
 <211> 642  
 10 <212> DNA  
 <213> Homo sapiens

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 15 ggagggggcg gaagcggcgg agggggggac acccgaccac gtttcttga gcaggttaa 120  
 catgagtgtc atttctcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
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 gccgcggtgg acacctactg cagacacaac tacggggttg gtgagagctt cacagtgcag 360  
 20 cggcgaacta gtggtggcgg tggcagcatc aaagaagaac atgtgatcat ccaggccgag 420  
 ttctatctga atcctgacca atcaggcgag ttatgtttg actttgatgg tgatgagatt 480  
 ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 540  
 gccagctttg aggtcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600  
 atcatgacaa agcgtccaa ctatactccg atcaccaatt aa 642

25 <210> 33 CO582  
 <211> 651  
 <212> DNA  
 <213> Homo sapiens

30 <400> 33  
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 catgagtgtc atttctcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 35 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
 ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300  
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 caggccgagt tctatctgaa tcctgaccaa tcaggcgagt ttatgtttga ctttgatggt 480  
 40 gatgagattt tccatgtgga tatggcaaag aaggagacgg tctggcggct tgaagaattt 540  
 ggacgatttg ccagcttga ggctcaagg gcatggcca acatagctgt ggacaaagcc 600  
 aacctggaaa tcatgacaaa gcgtccaaac tatactccga tcaccaatta a 651

45 <210> 34 CO581  
 <211> 651  
 <212> DNA  
 <213> Homo sapiens

<400> 34  
 50 atgggggaca ccggtcgcag cttcacctg gcctccagcg agaccggcgt gggcgctagc 60

ggagggggcg gaagcggcgg agggggggac acccgaccac gtttcttga gcaggttaa 120  
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 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
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 5 gccgcggtgg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360  
 cggcgaggct ctctcctg tggaccacct ggatctatca aagaagaaca tgtgatcatc 420  
 caggccgagt tctatctgaa tcctgaccaa tcaggcgagt ttatgttga ctttgatggt 480  
 gatgagattt tccatgtgga tatggcaaag aaggagacgg tctggcggt tgaagaattt 540  
 ggacgatttg ccagcttga ggctcaagg gcatggcca acatagctgt ggacaaagcc 600  
 10 aacctgga aaatgacaaa gcgtccaac tatactcca tcaccaatta a 651

<210> 35 CO580

<211> 663

<212> DNA

15 <213> Homo sapiens

<400> 35

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 20 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
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 gccgcggtgg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360  
 cggcgaggga gtccaggcgg tggcgggagc ggcggtggtc ctgggagtat caaagaagaa 420  
 25 catgtgatca tccaggccga gttctatctg aatcctgacc aatcaggcga gtttatgtt 480  
 gactttgatg gtgatgatg ttccatgtg gatatggcaa agaaggagac ggtctggcgg 540  
 cttgaagaat ttggacgatt tgccagctt gaggtcaag gtgcattggc caacatagct 600  
 gtggacaaag ccaacctgga aatcatgaca aagcgtcca actatactcc gatcaccaat 660  
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30

<210> 36 CO567

<211> 663

<212> DNA

<213> Homo sapiens

35

<400> 36

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 catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
 40 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240  
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 gccgcggtgg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360  
 cggcgaacta gtgtggcgg tggcagcggc ggtggtggt cctcgagtat caaagaagaa 420  
 catgtgatca tccaggccga gttctatctg aatcctgacc aatcaggcga gtttatgtt 480  
 45 gactttgatg gtgatgatg ttccatgtg gatatggcaa agaaggagac ggtctggcgg 540  
 cttgaagaat ttggacgatt tgccagctt gaggtcaag gtgcattggc caacatagct 600  
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50 <210> 37 CO598

<211> 213  
<212> PRT  
<213> Homo sapiens

5 <400> 37  
Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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10 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Asp Thr Arg  
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
35 40 45

15 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
65 70 75 80

20 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
100 105 110

25 Val Gly Glu Ser Phe Thr Val Leu Arg Arg Leu Gly Gly Glu Asp Asp  
115 120 125

30 Glu Ala Asp His His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro  
130 135 140

Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe  
145 150 155 160

35 His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe  
165 170 175

Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala  
180 185 190

40 Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr  
195 200 205

45 Pro Ile Thr Asn  
210

<210> 38 CO597  
50 <211> 213

<212> PRT

<213> Homo sapiens

<400> 38

5 Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
20 25 30

10 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
35 40 45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
15 50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
65 70 75 80

20 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
100 105 110

25 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Ser Gly Gly  
115 120 125

Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro  
30 130 135 140

Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe  
145 150 155 160

35 His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe  
165 170 175

Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala  
180 185 190

40 Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr  
195 200 205

Pro Ile Thr Asn  
45 210

<210> 39 CO596

<211> 212

50 <212> PRT

<213> Homo sapiens

<400> 39

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly

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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Asp Thr Arg

20                    25                    30

10 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly

35                    40                    45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu

50                    55                    60

15

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu

65                    70                    75                    80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu

20                    85                    90                    95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly

100                    105                    110

25 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Ser Ile

115                    120                    125

Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp

130                    135                    140

30

Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His

145                    150                    155                    160

Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly

35                    165                    170                    175

Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val

180                    185                    190

40 Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro

195                    200                    205

Ile Thr Asn

210

45

<210> 40 CO595

<211> 211

<212> PRT

50 <213> Homo sapiens



<400> 40  
 Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
                   20                  25                  30  
 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
 10                  35                  40                  45  
 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
                   50                  55                  60  
 15 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
                   65                  70                  75                  80  
 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
                   85                  90                  95  
 20 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
                   100                  105                  110  
 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Gly Ile Lys  
 25                  115                  120                  125  
 Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln  
                   130                  135                  140  
 30 Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val  
                   145                  150                  155                  160  
 Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg  
                   165                  170                  175  
 35 Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp  
                   180                  185                  190  
 Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile  
 40                  195                  200                  205  
 Thr Asn  
                   210  
 45  
 <210> 41 CO593  
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 <212> PRT  
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<400> 41

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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5 Val Gly Ala Ser Gly Gly Gly Ser Gly Gly Gly Asp Thr Arg  
20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
35 40 45

10 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
50 55 60

15 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
85 90 95

20 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Val Tyr Pro Glu Val Thr  
115 120 125

25 Val Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn  
130 135 140

30 Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile  
145 150 155 160

Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu  
165 170 175

35 Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile  
180 185 190

Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr  
195 200 205

40 Thr Pro Ile Thr Asn  
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45 <210> 42 CO585

<211> 215

<212> PRT

<213> Homo sapiens

50 <400> 42

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5 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Asp Thr Arg  
                   20                  25                  30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
                   35                  40                  45

10 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
                   50                  55                  60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
                   65                  70                  75                  80

15 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
                   85                  90                  95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
                   100                  105                  110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Phe Asp Ala Pro Ser Pro  
                   115                  120                  125

25 Leu Pro Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu  
                   130                  135                  140

Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu  
                   145                  150                  155                  160

30 Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu  
                   165                  170                  175

Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn  
                   180                  185                  190

35 Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn  
                   195                  200                  205

40 Tyr Thr Pro Ile Thr Asn  
                   210

<210> 43 CO584  
 45 <211> 214  
       <212> PRT  
       <213> Homo sapiens

<400> 43  
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	Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg		
	20	25	30
5	Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly		
	35	40	45
	Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu		
10	50	55	60
	Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu		
	65	70	75 80
15	Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu		
	85	90	95
	Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly		
	100	105	110
20	Val Gly Glu Ser Phe Thr Val Gln Arg Arg Ser Gly Gly Ser Gly Gly		
	115	120	125
	Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn		
25	130	135	140
	Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile		
	145	150	155 160
30	Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu		
	165	170	175
	Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile		
	180	185	190
35	Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr		
	195	200	205
	Thr Pro Ile Thr Asn		
40	210		
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10 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
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15 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
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Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Gly Gly Gly  
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Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe  
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	Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu		
	85	90	95
15	Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly		
	100	105	110
	Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Pro Gly Gly		
	115	120	125
20	Pro Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe		
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	Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly		
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           35              40              45

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Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
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10 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
       85              90              95

15 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
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Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Gly Gly Gly  
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20 Gly Ser Gly Gly Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile  
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Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe  
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Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala  
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Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile  
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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
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50



Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
 35 40 45

5 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
 50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
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10 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
 85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
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15 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr Ser Gly Gly Gly Gly  
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Ser Gly Gly Gly Gly Ser Ser Ser Ile Lys Glu Glu His Val Ile Ile  
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Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe  
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30 Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile  
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Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
 35 210 215 220

## IMMUNE MEDIATORS AND RELATED METHODS

### ABSTRACT OF THE DISCLOSURE

The present invention is directed to recombinant DNA constructs that encode soluble, fused MHC heterodimers that may or may not be further linked to an antigenic  
5 peptide.

SF 1079183 v1

exhibit B O

09058-00565065

## FILING RECEIPT



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60/204,249	05/15/2000		150	14058-005650US	8		

20350  
TOWNSEND AND TOWNSEND AND CREW LLP  
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Date Mailed: 08/03/2000

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## Applicant(s)

Zhu Shirley, Residence Not Provided;

## Continuing Data as Claimed by Applicant

## Foreign Applications

If Required, Foreign Filing License Granted 08/02/2000

## Title

Immune mediators and related methods

## Preliminary Class

Data entry by : WASHINGTON, JAMES

Team : OIPE

Date: 08/03/2000



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Attorney Docket No. 14058-005650US"Express Mail" Label No. EL394880176USDate of Deposit: May 15, 2000

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Washington, D.C. 20231

Sir:

Transmitted herewith for filing is a provisional patent application under CFR 1.53(c) of:

LAST NAME	FIRST NAME	MIDDLE INITIAL	RESIDENCE (City/State/Country)
Shirley	Zhu		

Title: IMMUNE MEDIATORS AND RELATED METHODS

Enclosed are:

☒ 4 pages of the specification (including description and claims).☒ 8 sheet(s) of [ ] formal [ ☒ ] informal drawing(s).☒ 3 Sheets of Tables.

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SF 1096746 v1

Respectfully submitted,

TOWNSEND and TOWNSEND and CREW LLP

  
Kevin Bastian

Reg. No. 34,774

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PROVISIONAL

PATENT APPLICATION

IMMUNE MEDIATORS AND RELATED METHODS

Inventor(s):

Shirley Zhu

Assignee:

Corixa Corporation  
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Seattle, WA 98104

Entity:

## IMMUNE MEDIATORS AND RELATED METHODS

## CROSS-REFERENCES TO RELATED APPLICATIONS

The present application is related to U.S. Serial No. 09/261811, filed  
5 March 3, 1999, which is a continuation-in-part of U.S. Serial No. 08/480,002, filed June  
7, 1995, now abandoned, U.S. Serial No 08/657,581 filed June 7, 1996, now abandoned  
and U.S. Serial No 09/184,692 November 2, 1998, all of which are incorporated herein by  
reference.

## SUMMARY OF THE INVENTION

10 There is currently a great interest in developing pharmaceuticals based on  
the growing understanding of the structure and function of the major histocompatibility  
complex (MHC) antigens. These cell surface glycoproteins are known to play an  
important role in antigen presentation and in eliciting a variety of T cell responses to  
antigens. Identification of synthetic antigenic peptides, and demonstration that these  
15 peptides bind selectively to MHC molecules associated with disease and that stimulates T  
cells would help to implicate a particular peptide or peptide:MHC complex in  
susceptibility to an autoimmune disease. In particular, the development of soluble, fused  
MHC heterodimer:peptide complexes would be particularly useful in treatment of a  
number of diseases associated with antigen presentation by MHC molecules.

20 The present invention provides recombinant DNA constructs that encode  
soluble, fused MHC heterodimers that may or may not be further linked to an antigenic  
peptide. Typically, the constructs comprise a first DNA segment encoding at least a  
portion of a first domain of a selected MHC molecule; a second DNA segment encoding  
at least a portion of a second domain of the selected MHC molecule; a first linker DNA  
25 segment and connecting in-frame the first and second DNA segments; wherein linkage of  
the first DNA segment to the second DNA segment by the first linker DNA segment  
results in a fused first DNA-first linker-second DNA polysegment. The constructs of the  
invention may also comprise a third DNA segment encoding an antigenic peptide capable  
of associating with a peptide binding groove of the selected MHC molecule and a second  
30 linker DNA segment connecting in-frame the third DNA segment to the fused first DNA-  
first linker-second DNA polysegment.

The present invention provides soluble-fused heterodimers that comprise an  
additional polypeptide sequence that allows for inter-chain dimerization of the  
heterodimers of the invention. The sequence can be any sequence that allows for

covalent or non-covalent linkages between the molecules of the invention. As shown below, a preferred means for carrying this out is through use of segments from immunoglobulin family proteins (*e.g.*, antibodies, MHC molecules, T cell receptors and the like) that have cysteine residues capable of forming interchain disulfide bonds. An example shown below is the use of the constant region of the Kappa chain of an antibody. One of skill will recognize that any of a number of polypeptide sequences can be used for this purpose.

In the present study, monomeric and dimeric forms of recombinant I-AS-peptide complexes, fused to an antigenic MBP 90-101 peptide with flexible linkers were constructed. The recombinant I-AS proteins share structural similarity to that of crystallized native MHC class II protein by protein modeling. The recombinant proteins were expressed in *E. coli* and in an insect expression system and purified by affinity chromatography and FPLC. The purified recombinant I-AS proteins showed *in vitro* biological activity as assayed using an antigen-specific mouse T cell clone. The *in vivo* activity of the recombinant I-AS fusion proteins in the EAE model using susceptible SJL mice shows that treatment with the recombinant I-AS proteins prevents mortality and significantly reduces paralysis induced by myelin homogenate. Histological examination of sections from animal spinal cord reveals that these treatments also reduce the inflammatory lesions. In conclusion, these studies suggest that the recombinant I-AS fusion proteins have therapeutic benefit as antigen-specific drugs for the treatment of autoimmune diseases.

Figure 1. Structure of the recombinant I-AS .MBP. $\beta$ 1 $\alpha$ 1 (monomer) and I-AS.MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CK (dimer) proteins

Figure 2. Structural comparison of the recombinant I-AS proteins with the crystallized native murine MHC class II protein, I-EK. White color: murine MHC class II, I-EK, Yellow color: murine MHC class II, I-AS, Green color: antigenic peptide, Red color: linkers between antigenic peptide and  $\beta$ 1 domain of I-AS or between  $\beta$ 2 and  $\alpha$ 1 domains of I-AS or between  $\alpha$ 2 and IgG Ck fragment. Blue color: IgG Ck domains. The alpha helix and beta sheets folded from the both I-AS fusion proteins are very similar to the crystallized native murine MHC class II, I-EK. The I-AS.MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CK protein forms a dimer.

Figure 3. Silver staining and Western analysis of the purified recombinant I-AS monomer and dimer. Upper left, silver stained gel with and without MBP 90-101 peptide fusion of monomeric I-AS proteins. Upper-middle and upper-right,



Western analysis of monomeric I-AS proteins blotted with anti murine MHC class II protein, 10-2.16 and anti-MBP 90-101. Lower-left, silver-stained gel of purified dimeric I-AS protein. Lower-middle, Western analysis blotted with goat anti mouse IgG specific to the Ck region. Lower-right, glycosylation studies of the dimer molecules expressed in insect cells blotted with goat anti-mouse IgG specific to the Ck region.

Table 1. Summary of recombinant I-AS protein expression and purification in *E. coli* and Baculo-viral infected high 5 cells.

Figure 4. Diagram of the in vitro biological activity assay for the recombinant I-AS proteins.

Figure 5 In vitro biological activities of the recombinant I-AS proteins compared with APC+ antigenic peptide in the mouse T cell clone, HS-1. A. I-AS.MBP. $\beta$ 1 $\alpha$ 1, monomer, B. I-AS.MBP.Ck, dimer, and C. APC+ antigenic peptide. The positive (anti-CD3) and the negative (HS-1 cell alone) controls are also shown in each panel.

Figure 6. Diagram of the EAE model and standard for EAE scoring.

Figure 7. The EAE model studies. The recombinant I-AS proteins were administered to SJL mice on day 1, 4, 7, and 11 by i.v. injection after inducing the disease with myelin emulsified in CFA. The animals were evaluated for neurological dysfunction. Panel A: Untreated, injected with same amount of PBS solution. Panel B. Treated with the recombinant I-AS.MBP.Ck protein, a dimer form. Panel C: Treated with the recombinant I-AS. $\beta$ 1 $\alpha$ 1. This recombinant molecule does not carry the antigenic peptide. Panel D: Treated with the recombinant I-AS.MBP. $\beta$ 1 $\alpha$ 1, a monomer form.

Table 2. Summary of reduction of EAE incidences in SJL mice by administration of recombinant I-AS proteins.

Figure 8. The representative spinal cord sections of histological examination from SJL mice in the EAE model studies. Spinal cords were removed, fixed in formalin solution and embedded in paraffin. Sections were cut, stained with hemotoxylin, eosin and graded for inflammatory lesions. A. Section of spinal cord from untreated mouse; B. Section of spinal cord from mouse treated with the recombinant I-AS.MBP.Ck; C. Section of spinal cord from mouse treated with the recombinant I-AS. $\beta$ 1 $\alpha$ 1 without antigenic peptide fusion; D. Section of spinal cord from mouse treated with the recombinant I-AS.MBP. $\beta$ 1 $\alpha$ 1. The histology score for each section is marked.

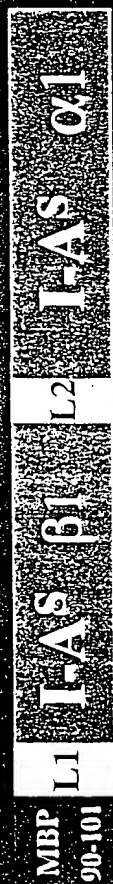
Table 3. Summary of histology data from spinal cord sections.

The amino acid sequence of the I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.C $\kappa$  shown in Figure 1 is as follows:

>

METDTLLLWLLLWPGSTGDFKNIVTPRTPPPASGGGGSGGGGDSERHFVFQFKGECYFTNGTQRI  
RSVDRIYINREEYLRFDSVDGEYRAVTELGRPDPYINKQYLEQTRAELDTVCRHNYEGVETHTSLR  
RLEQPNVVISLSRTEALNHHNTLVCSVTDFYPAKIKVRWFRNGQEETVGVSSSTQLIRNGDWTFQVLVM  
LEMTPRRGEVYTCHVEHPSLKSPITVEWTSGGGGSGGGGSGGGGSGGGGSSSEDDIEADHVGVIY  
TTVYQSPGDIGQYTHEFDGDEWIFYVLDKKETIWMLPEFGQLTSFDPQGGLQNIATGKYTLGILTKRS  
NSTPATNEAPQATVFPKSPVLLGQPNTLICFVDNIFPPVINITWLRNSKSVTDGVYETSFLVNRDHSFHK  
LSYLTIFPSDDDIYDCKVEHWGLEEPVLKHWASGGGGSGGGGADAAPTVSIFPPSSEQLTSGGASV  
CFLNNFYPKDINVKWKIDGSRQNGVLNSWTDQDSKSTYSMSSTLTLTKEYERHNSYTCEATHKT  
STSPIVKSFNRECE.

SF 1095899 v1



mIgGyl

mIgGyl

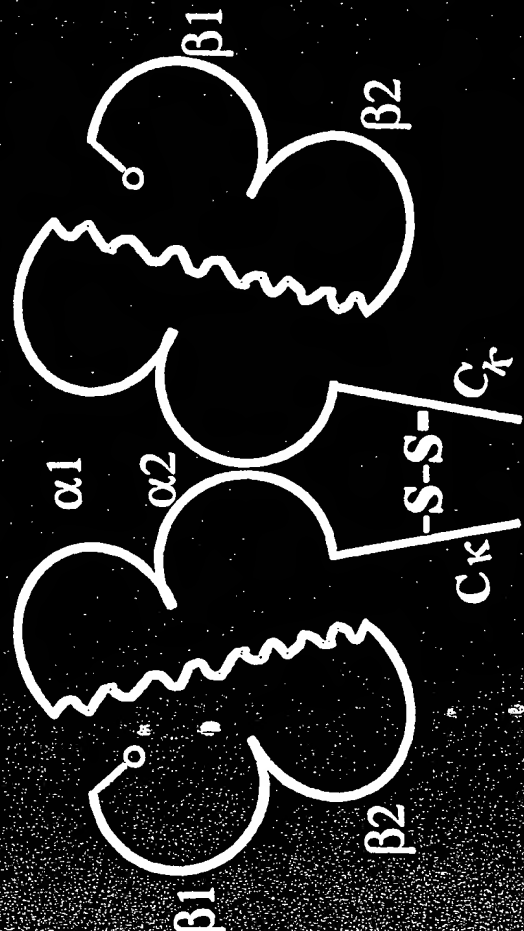


I-A<sup>s</sup>MBP.β1β2α1α2.Ck

I-A<sup>s</sup>MBP.β1α1



Monomer



Dimer

Figure 1

**I-EK-HSP70**



**I-ASMBP $\beta$ 1 $\alpha$ 1**  
(Monomer)



**I-ASMBP**  
 **$\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck**  
(Dimer)



David H. Fremont, Wayne A. Hendrickson  
Phillippa Marrack, John Kappler  
SCIENCE, 1996, 272:1001-1004

Figure 2

# Silver Staining and Western Analysis of Recombinant I-A<sup>S</sup>.MBP.β1α1 Proteins

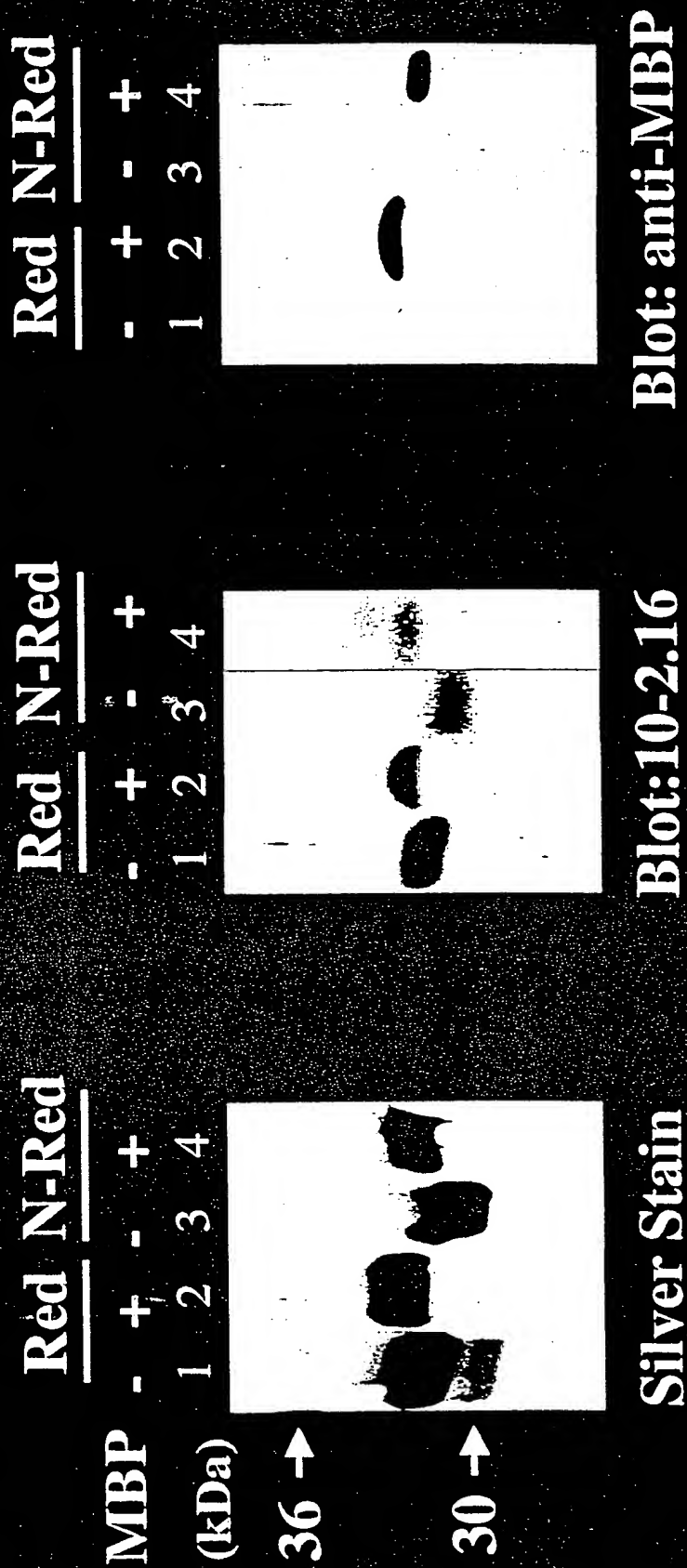


Figure 3

**Table 1. Summary of Recombinant I-A<sup>S</sup> Protein Expression and Purification**

Protein	Expression System	Protein localization	Expression Level	Method for Purification
I-A <sup>S</sup> MBP $\beta$ 1 $\alpha$ 1	E.coli	Inclusion Body	>100 mg/L	FPLC: ion-EX & size exclusion
I-A <sup>S</sup> MBP Ck	Baculo-High 5	Secreted form	20-25 mg/L	IgG affinity Chromatography

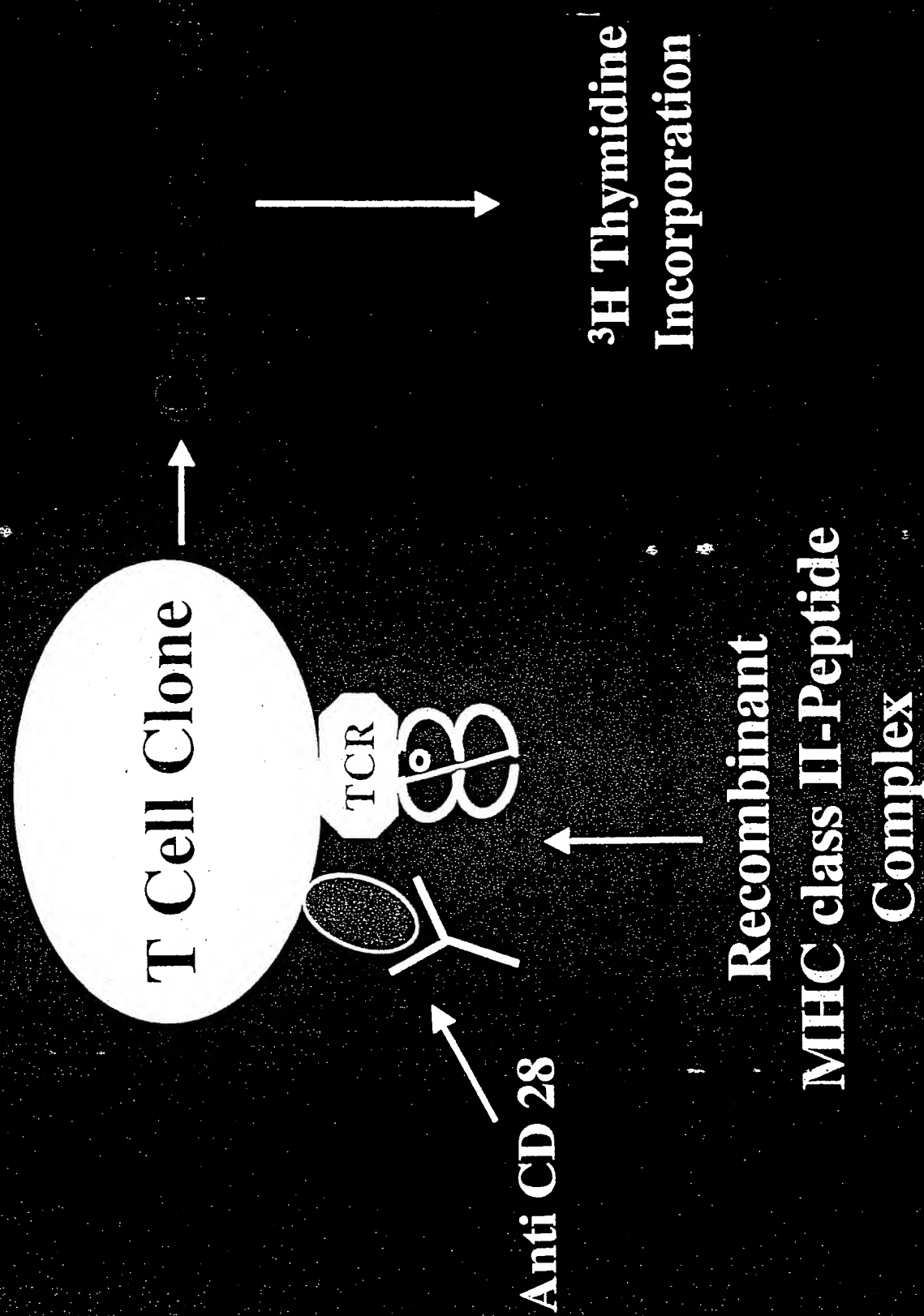
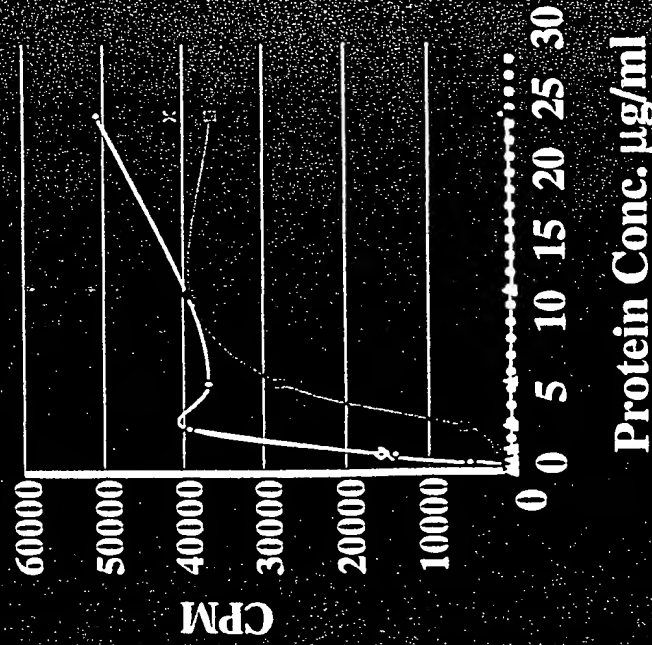


Figure 4

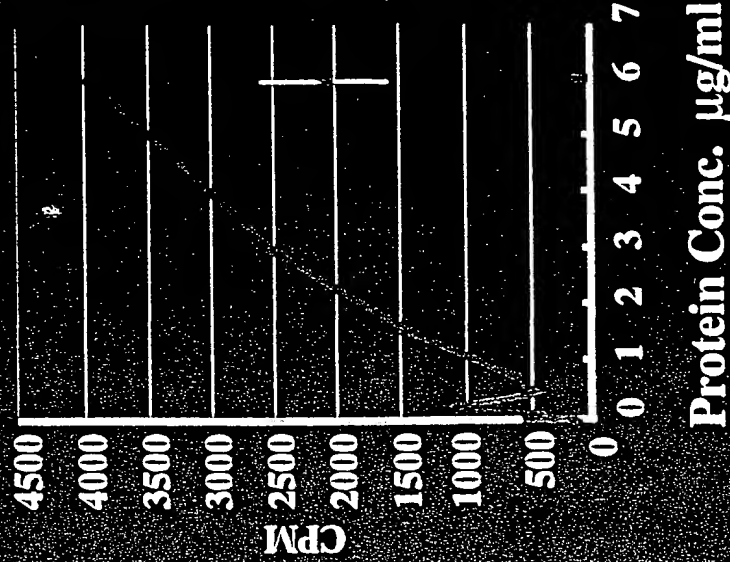


-- IAs.MBP. $\beta$ 1 $\alpha$ 1  
 APC+MBP(90-101)  
 -- IAs. $\beta$ 1 $\alpha$ 1  
 anti-CD3  
 APC alone  
 HS-1 alone



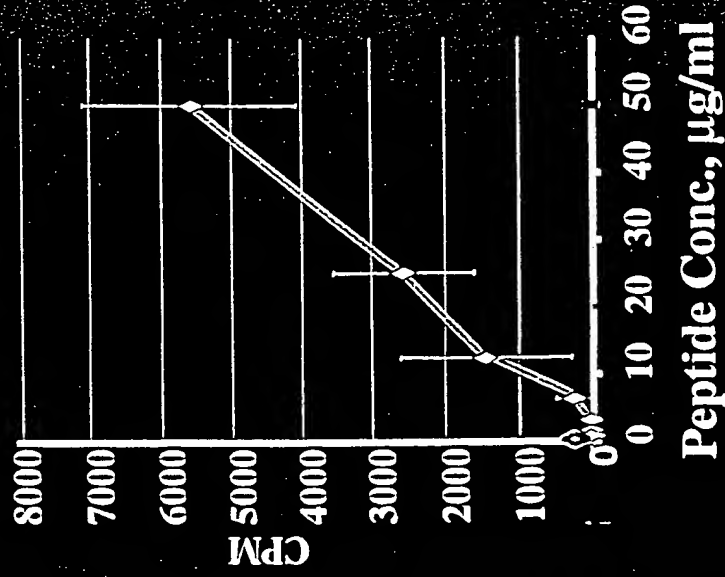
A

IAs.MBP.Ck  
 HS-1 alone  
 Anti-CD3



B

-- APC + Peptide  
 APC alone



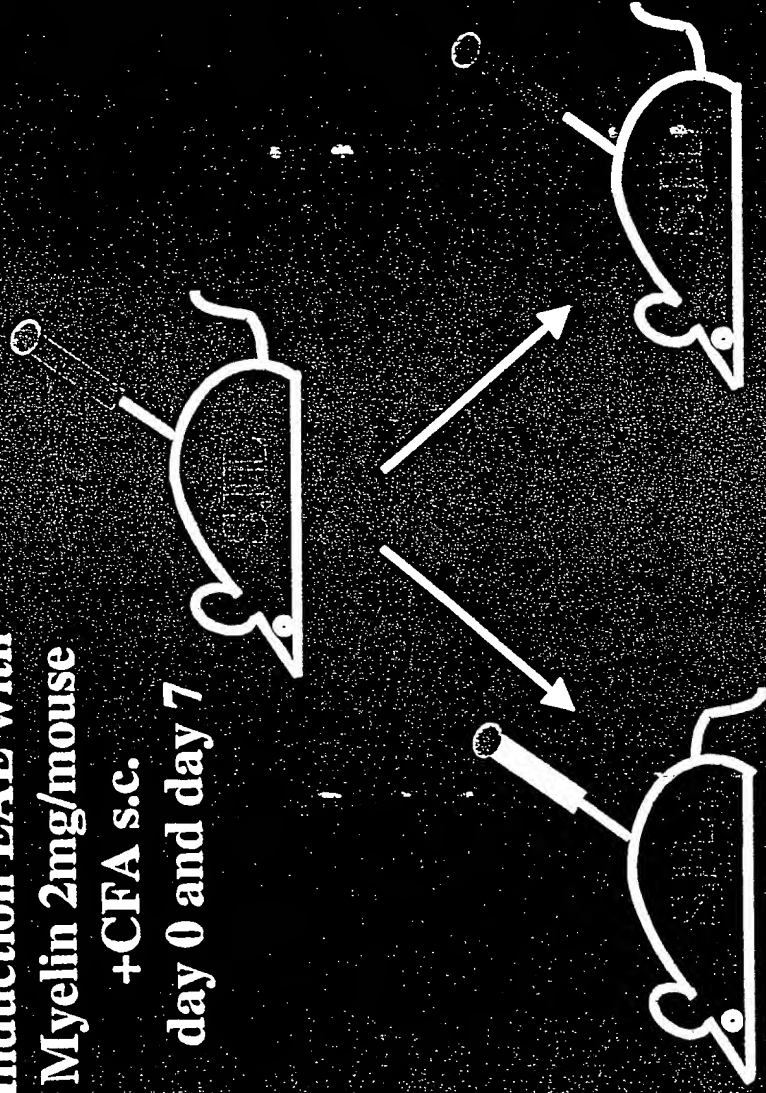
C

Figure 5



**Induction EAE with  
Myelin 2mg/mouse  
+CFA s.c.**

**day 0 and day 7**



**Untreated  
none/PBS**

**Recombinant I-A.S.MBP  
i.v. 10 µg/mouse**

**Inject at day 1, 4, 7, and 11  
Monitoring EAE at week 2-8**

## **EAE Score**

**0: Normal**

**0.5: Stiff tail**

**1.0: Limp tail**

**1.5: Limp tail with  
impaired righting reflex**

**2.0: Paralysis of one limb**

**2.5: Paralysis of one limb  
and weakness of the other**

**3.0: Complete paralysis  
of both hind limbs**

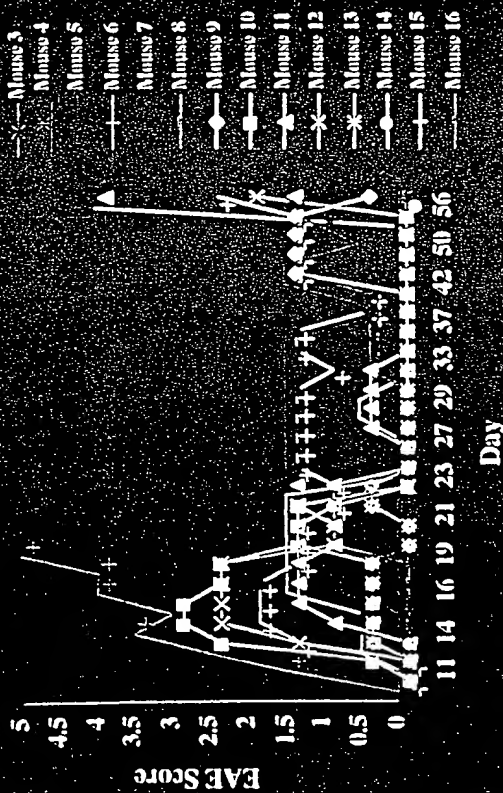
**4.0: Moribund**

**5.0: Death**

**Figure 6**

# A

## Untreated



# B

## I-ASMBP.Ck



# C

## I-AS $\beta$ 1 $\alpha$ 1



# D

## I-ASMBP $\beta$ 1 $\alpha$ 1

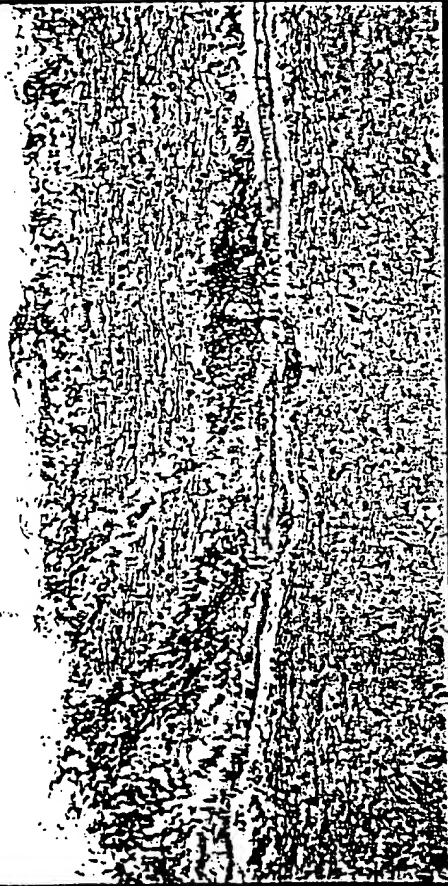


Figure 7

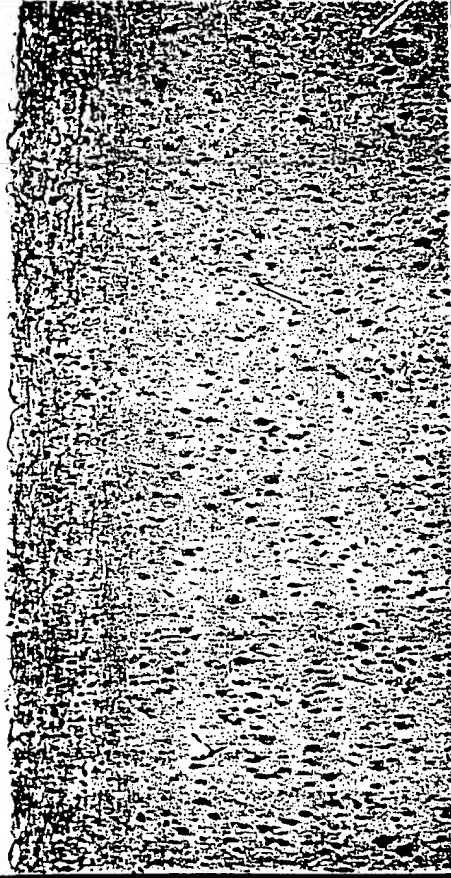
**Table 2. Summary of Reduction of EAE in SJL mice  
by Administration of Recombinant I-A<sup>S</sup> Proteins**

Treatment	Mice with disease
myelin/CFA+ none/ PBS	45/76 (59.2%)
myelin/CFA+ IAs.MBP.β1α1	7/56 (12.5%)
myelin/CFA + IAs.MBP.Ck	4/28 (14.3%)
myelin/CFA + IAs.β1α1	16/32 (50.0%)

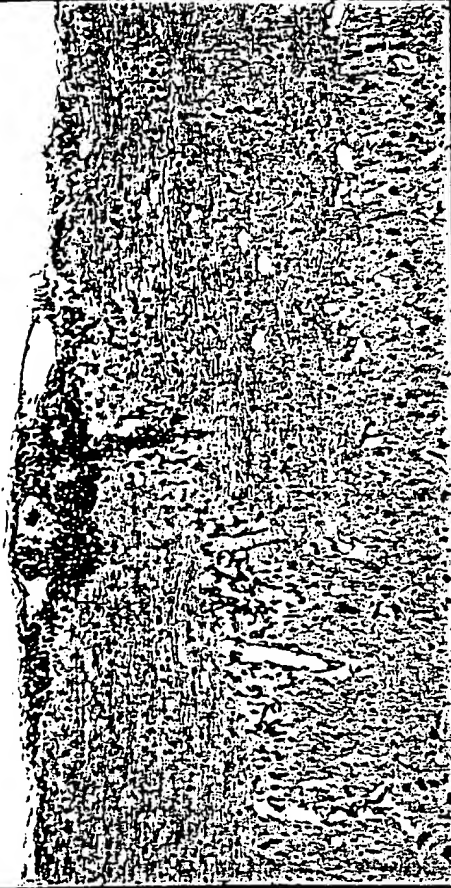
**A.** Untreated, histology score: 2.5



**B.** I-A<sup>S</sup>.MBP.Ck, histology score: 0



**C.** I-A<sup>S</sup>. $\beta$ 1 $\alpha$ 1, histology score: 2.0



**D.** I-A<sup>S</sup>.MBP. $\beta$ 1 $\alpha$ 1, histology score: 0.5

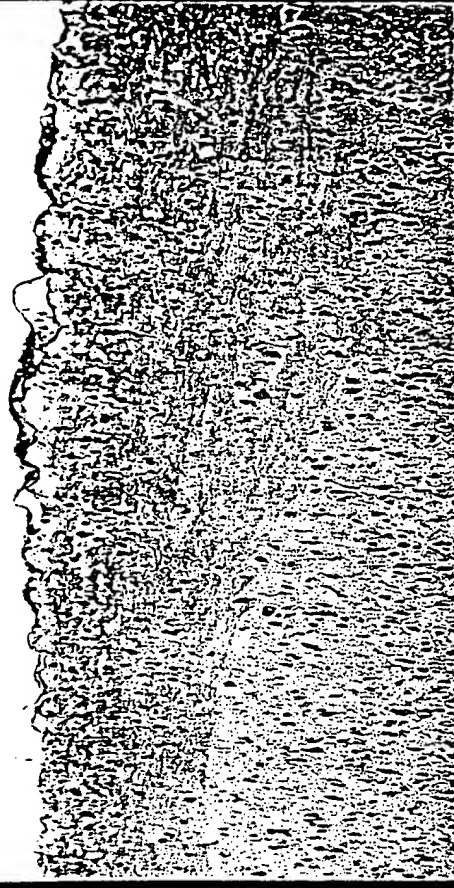


Figure 8



0 Exhibit

014058-605660US

KL Bastian

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## Foreign Applications

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## Title

Immune mediators and related methods

## Preliminary Class

Data entry by : PROCTOR, DARLENE

Team : PCT

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Cx-00-0200

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Cx-00-0359

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**PROVISIONAL**

**PATENT APPLICATION**

**IMMUNE MEDIATORS AND RELATED METHODS**

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## IMMUNE MEDIATORS AND RELATED METHODS

### CROSS-REFERENCES TO RELATED APPLICATIONS

This application is related to U.S. Serial No. 60/191,274, filed March 22,  
5 2000; and U.S. Serial No. 60/204,249, filed May 15, 2000. Each of the aforementioned  
applications are herein incorporated by reference in their entirety.

This application is also related to U.S. Serial No. 09/261,811, filed March  
3, 1999; which is a continuation of U.S. Serial No. 08/657,581, filed June 7, 1996, now  
abandoned; which is a continuation in part of U.S. Serial No. 08/480,002, filed June 7,  
10 1995, now abandoned; U.S. Serial No 09/184,692, filed November 2, 1998, now  
abandoned; U.S. Serial No. 08/483,241, filed June 7, 1995; U.S. Serial No. 08/482,133,  
filed June 7, 1995; and U.S. Provisional Application No. 60/005,964, filed October 27,  
1995.

### 15 BACKGROUND OF THE INVENTION

T cells, unlike B cells, do not directly recognize antigens. Instead, an  
accessory cell must first process an antigen and present it in association with an MHC  
molecule in order to elicit a T cell-mediated immunological response. The major function  
of MHC glycoproteins appears to be the binding and presentation of processed antigen in  
20 the form of short antigenic peptides.

In addition to binding foreign or "non-self antigenic peptides," MHC  
molecules can also bind "self" peptides. If T lymphocytes then respond to cells  
presenting "self" or autoantigenic peptides, a condition of autoimmunity results. Over 30  
autoimmune diseases are presently known, including myasthenia gravis (MG), multiple  
25 sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), insulin-  
dependent diabetes mellitus (IDDM), etc. Characteristic of these diseases is an attack by  
the immune system on the tissues of the host. In non-diseased individuals, such attack  
does not occur because the immune system recognizes these tissues as "self."  
Autoimmunity occurs when a specific adaptive immune response is mounted against self  
30 tissue antigens.

There is therefore currently a great interest in developing pharmaceuticals  
based on the growing understanding of the structure and function of the major  
histocompatibility complex (MHC) antigens. Identification of synthetic autoantigenic

peptides, and demonstration that these peptides bind selectively to MHC molecules associated with disease and that stimulates T cells would help to implicate a particular peptide or peptide:MHC complex in susceptibility to an autoimmune disease. In particular, the development of soluble, fused MHC heterodimer:peptide complexes would be particularly useful in treatment of a number of diseases associated with antigen presentation by MHC molecules. Furthermore, the development of single chain, multimeric complexes would be of interest (*see, e.g.*, WO 93/10220, WO 98/05684, WO 97/35991, WO 98/03552, WO 99/13095, WO 98/06749, WO 99/09064, and U.S. Patent 5,869,270).

## SUMMARY OF THE INVENTION

The present invention provides recombinant nucleic acid constructs that encode single chain, recombinant MHC class II heterodimers (e.g., "soluble, fused heterodimers") that may or may not be further linked to an antigenic peptide. The constructs of the invention are dimerized or multimerized by inter-chain dimerization or multimerization of the single chain heterodimers of the invention. The dimerization or multimerization sequence can be any sequence that allows for covalent or non-covalent linkages between the molecules of the invention, e.g., by direct chemical means or by recombinant means. As shown below, a preferred means for carrying this out is through use of segments from immunoglobulin family proteins (e.g., antibodies, MHC molecules, T cell receptors and the like) that have cysteine residues capable of forming interchain disulfide bonds. In another embodiment, a leucine zipper domain forms a non-covalent linkage. An example shown below is the use of the constant region of the kappa chain of an antibody. One of skill will recognize that any of a number of polypeptide sequences can be used for this purpose. The single chain molecules of the invention thus can be multimers wherein each single chain heterodimer is from a different MHC class II allele. In addition, each heterodimer in the multimer can be bound to a different antigen.

In one embodiment, monomeric and dimeric forms of recombinant mouse I-AS-peptide complexes, fused to an antigenic MBP 90-101 peptide with flexible linkers were constructed. The recombinant I-AS proteins share structural similarity to that of crystallized native human MHC class II protein as determined by protein modeling. The recombinant proteins were expressed in *E. coli* and in an insect expression system and

purified by affinity chromatography and FPLC. The purified recombinant I-AS proteins showed *in vitro* biological activity as assayed using an antigen-specific mouse T cell clone. The *in vivo* activity of the recombinant I-AS fusion proteins in the EAE model using susceptible SJL mice shows that treatment with the recombinant I-AS proteins prevents mortality and significantly reduces paralysis induced by myelin homogenate. Histological examination of sections from animal spinal cord reveals that these treatments also reduce the inflammatory lesions. These results demonstrate that the single chain MHC class II heterodimers have therapeutic benefit as antigen-specific drugs for the treatment of autoimmune diseases.

In another embodiment, novel linkers are provided for forming single chain MHC class II heterodimers. These linkers can be used with the multimer constructs described above. In another embodiment, the constructs of the invention are optimized for prokaryotic expression, using codons adjusted for *E. coli* codon bias.

#### BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Schematic structure of the recombinant I-AS .MBP. $\beta$ 1 $\alpha$ 1 (monomer) and I-AS.MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CK (dimer) proteins.

Figure 2. Structural comparison of the recombinant I-AS proteins with the crystallized native murine MHC class II protein, I-EK. White color: murine MHC class II, I-EK, Yellow color: murine MHC class II, I-AS , Green color: antigenic peptide, Red color: linkers between antigenic peptide and  $\beta$ 1 domain of I-AS or between  $\beta$ 2 and  $\alpha$ 1 domains of I-AS or between  $\alpha$ 2 and IgG Ck fragment. Blue color: IgG Ck domains. The tertiary structure predicted for both I-AS fusion proteins are very similar to the crystallized native murine MHC class II, I-EK. The I-AS.MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CK protein can form a dimer.

Figure 3. Silver staining and western analysis of the purified recombinant I-AS monomer and dimer. Left, silver stained gel with and without MBP 90-101 peptide fusion of monomeric I-AS proteins. Middle, western analysis of monomeric I-AS proteins blotted with anti murine MHC class II protein, 10-2.16. Right, western analysis of monomeric I-AS proteins blotted anti-MBP 90-101.

Figure 4. Diagram of the *in vitro* biological activity assay for the recombinant I-AS proteins.

Figure 5. *In vitro* biological activities of the recombinant I-AS proteins compared with APC+ antigenic peptide in the mouse T cell clone, HS-1.

A. I-AS.MBP. $\beta$ 1 $\alpha$ 1, monomer, B. I-AS.MBP.Ck, dimer, and C. APC+ antigenic peptide. The positive (anti-CD3) and the negative (HS-1 cell alone) controls are also shown in each panel.

Figure 6. Diagram of the EAE model and standard for EAE scoring.

Figure 7. The EAE model studies. The recombinant I-AS proteins were administered to SJL mice on day 1, 4, 7, and 11 by i.v. injection after inducing the disease with myelin emulsified in CFA. The animals were evaluated for neurological dysfunction. Panel A: Untreated, injected with same amount of PBS solution. Panel B: Treated with the recombinant I-AS.MBP.Ck protein, a dimer form. Panel C: Treated with the recombinant I-AS. $\beta$ 1 $\alpha$ 1. This recombinant molecule does not carry the antigenic peptide. Panel D: Treated with the recombinant I-AS.MBP. $\beta$ 1 $\alpha$ 1, a monomer form.

Figure 8. The representative spinal cord sections of histological examination from SJL mice in the EAE model studies. Spinal cords were removed, fixed in formalin solution and embedded in paraffin. Sections were cut, stained with hematoxylin, eosin and graded for inflammatory lesions. A. Section of spinal cord from untreated mouse; B. Section of spinal cord from mouse treated with the recombinant I-AS.MBP.Ck; C. Section of spinal cord from mouse treated with the recombinant I-AS. $\beta$ 1 $\alpha$ 1 without antigenic peptide fusion; D. Section of spinal cord from mouse treated with the recombinant I-AS.MBP. $\beta$ 1 $\alpha$ 1. The histology score for each section is marked.

Figure 9 shows a schematic representation of a truncated single chain MHC class II peptide complex that is a dimer with two peptide specificities.

Figure 10 shows a schematic representation of a single chain MHC class II peptide complex that is a dimer with two peptide specificities.

Figure 11 shows a schematic representation of an single chain MHC class II peptide complex that is a tetramer with four peptide specificities.

Figure 12 shows a schematic representation of an single chain MHC class II peptide complex that is a tetramer with two peptide specificities and two different MHC class II alleles.

Figure 13 shows the effect of different recombinant MHC class II molecules on the development of EAD (day 60+).

## DETAILED DESCRIPTION OF THE INVENTION

**Introduction**

The present invention provides recombinant DNA constructs that encode  
5 soluble, fused MHC class II heterodimers (i.e., single chain MHC class II heterodimers)  
that may or may not be further linked to an antigenic peptide. Typically, the constructs  
comprise a first DNA segment encoding a  $\beta$ 1 domain of a selected MHC class II  
molecule; a second DNA segment encoding a  $\alpha$ 1 domain of the selected MHC class II  
molecule; and a first linker DNA segment connecting in-frame the first and second DNA  
10 segments; wherein linkage of the first DNA segment to the second DNA segment by the  
first linker DNA segment results in a fused first DNA-first linker-second DNA  
polysegment. The constructs of the invention may also comprise a third DNA segment  
encoding an antigenic peptide capable of associating with a peptide binding groove of the  
selected MHC class II molecule and a second linker DNA segment connecting in-frame  
15 the third DNA segment to the fused first DNA-first linker-second DNA polysegment.

The present invention provides soluble-fused heterodimers that comprise  
an additional polypeptide sequence that allows for inter-chain dimerization of the  
heterodimers of the invention. The additional polypeptide allows multimerization of the  
single chain MHC class II molecules, to produce, e.g., dimers and tetramers. The  
20 sequence can be any sequence that allows for covalent or non-covalent linkages between  
the molecules of the invention. In one embodiment, the single chain molecules are  
covalently linked using chemical methods known to those of skill in the art, e.g.,  
photoaffinity methods or homo-bifunctional protein cross-linkers (*see, e.g., Hermanson et al., Bioconjugate Techniques*, (1996)). In one embodiment, the molecules are covalently  
25 linked using heterobifunctional protein cross-linkers. As shown below, one means for  
carrying this out is through use of segments from immunoglobulin family proteins (*e.g.,*  
antibodies, MHC molecules, T cell receptors and the like) that have cysteine residues  
capable of forming interchain disulfide bonds. An example shown below is the use of the  
constant region of the kappa chain of an antibody ( $C\kappa$ ), from either a heavy or a light  
30 chain. Other dimerization sequences include a leucine zipper, a STAT protein N-terminal  
domain, or the FK506 binding protein (*see, e.g., O'Shea, Science* 254: 539 (1991),  
Barahmand-Pour *et al., Curr. Top. Microbiol. Immunol.* 211:121-128 (1996); Klemm *et*

*al.*, *Annu. Rev. Immunol.* 16:569-592 (1998); Ho *et al.*, *Nature* 382:822-826 (1996)). One of skill will recognize that any of a number of polypeptide sequences can be used for this purpose.

In addition, the multimeric, single chain class II molecules of the invention  
 5 comprise at least two different MHC class II alleles that are associated with an autoimmune disease state, and/or at least two different autoantigenic peptides that are associated with a particular autoimmune disease state. In one example, the multimeric, single chain class II molecules have chains from different DR2 alleles, e.g., DRB5\*0101 and DRB1\*1501. In another embodiment, the autoantigenic peptides are peptides  
 10 associated with multiple sclerosis, e.g., MBP (e.g., amino acid residues 83-102Y83), PLP (e.g., amino acid residues 40-60, 89-106, 95-117, and 185-206); and MOG. In addition, other antigens associated with autoimmune disease, such as acetylcholine receptor and type II collagen, can be linked to the single chain heterodimers of the invention..

In a further embodiment, the single chain class II molecules of the  
 15 invention have novel linkers, as described herein. The mammalian MHC class II single chain constructs of the invention may also be constructed to use preferred prokaryotic codons, for expression, e.g., in *E. coli*, using codon preference tables and methods known to those of skill in the art.

## 20 Definitions

Prior to setting forth the invention, it may be helpful to an understanding thereof to provide definitions of certain terms to be used hereinafter:

Single chain MHC class II heterodimer: As used herein this term refers to a fusion protein such as the fused or single chain MHC class II heterodimer complex of  
 25 the invention, which optionally also is fused to a peptide to form a single chain MHC class II heterodimer:peptide complex. Such fusion proteins will be indicated with a colon (:). MHC-peptide complexes which are not fusion proteins, are native MHC containing protein or exogenously loaded MHC molecules are indicated with a dash (-). The fusion proteins of the invention can also be multimers, having two, four or more single chain  
 30 molecules linked covalently or non-covalently through multimerization domains in the single chain molecule. A single chain molecule of the invention typically comprises at

least an MHC class II  $\beta 1$  domain and an MHC class II  $\alpha 1$  domain, optionally  $\beta 1$   $\beta 2$   $\alpha 1$   $\alpha 2$  domains or any combination thereof in any order.

A domain of a selected MHC molecule: A portion of an MHC domain which is sufficient to form, either alone, or in combination with another portion of an MHC domain, a peptide binding site which is capable of presenting an antigenic peptide in such a fashion that it is recognized by a T cell receptor. Such MHC domains would include the extracellular portion of the two polypeptide chains of Class II MHC. This would include the  $\alpha$  chain ( $\alpha 1$  and  $\alpha 2$  domains) and  $\beta$  chain ( $\beta 1$  and  $\beta 2$  domains) of Class II MHC. This would include  $\beta 1$  and  $\alpha 1$ ,  $\beta 1$ ,  $\beta 2$  and  $\alpha 1$ ,  $\alpha 2$ ,  $\alpha 1$  or  $\alpha 2$  independent of the other, or  $\alpha 1$  and  $\alpha 2$  in tandem ( $\alpha 1\alpha 2$ ). It would also include  $\beta 1$  or  $\beta 2$  independent of the other, or  $\beta 1$  and  $\beta 2$  in tandem ( $\beta 1\beta 2$ ). This would also include any suitable combination of the  $\alpha 1$ ,  $\alpha 2$ ,  $\beta 1$ , and  $\beta 2$  domains. The domains can be directly linked, or can be linked via an amino acid linker.

Linker DNA segment: A segment of DNA encoding from about 1 to about 50, preferably from about 5 to about 25 amino acids, which forms a flexible link between two DNA segments. This flexible link allows the two DNA segments to attain a proper configuration, such as an MHC peptide binding groove, or allows a peptide to properly bind into such a groove.

Antigenic peptide: The immunological properties of MHC histocompatibility proteins are largely defined by the antigenic peptide that is bound to them. An antigenic peptide is one which contains an epitope (an amino acid sequence) recognized by immune cells, e.g., T cells, and is capable of stimulating an MHC-mediated immune response.. Antigenic peptides for a number of autoimmune diseases are known. For example, in experimentally induced autoimmune diseases, antigens involved in pathogenesis have been characterized: in arthritis in rat and mouse, native type II collagen is identified in collagen-induced arthritis, and mycobacterial heat shock protein in adjuvant arthritis (Stuart *et al.*, *Ann. Rev. Immunol.* 2:199-218, 1984; and van Eden *et al.*, *Nature* 331:171-173, 1988); thyroglobulin has been identified in experimental allergic thyroiditis (EAT) in mice (Marion *et al.*, *J. Exp. Med.* 152:1115-1120, 1988); acetylcholine receptor (AChR) in experimental allergic myasthenia gravis (EAMG) (Lindstrom *et al.*, *Adv. Immunol.* 42:233-284, 1988); and myelin basic protein (MBP) and proteolipid protein (PLP) in experimental allergic encephalomyelitis (EAE) in mouse and rat (Acha-

Orbea *et al.*, *Ann. Rev. Imm.* 7:377-405, 1989). In addition, target antigens have been identified in humans: type II collagen in human rheumatoid arthritis (Holoshitz *et al.*, *Lancet* ii:305-309, 1986), acetylcholine receptor in myasthenia gravis (Lindstrom *et al.*, *Adv. Immunol.* 42.:233-284, 1988), and MBP, PLP, and MOG in multiple sclerosis in humans.

**MHC:** The major histocompatibility complex (MHC) is a family of highly polymorphic proteins, divided into two classes, Class I and Class II, which are membrane-associated and present antigen to T lymphocytes (T cells). MHC Class I and Class II molecules are distinguished by the types of cells on which they are expressed, and by the subsets of T cells which recognize them. Class I MHC molecules (e.g., HLA-A, -B and -C molecules in the human system) are expressed on almost all nucleated cells and are recognized by cytotoxic T lymphocytes (CTL), which then destroy the antigen-bearing cells. Class II MHC molecules (HLA-DP, -DQ and -DR, for example, in humans) are expressed primarily on the surface of antigen-presenting cells, such as B lymphocytes, dendritic cells, macrophages, and the like. Class II MHC is recognized by CD4<sup>+</sup> T helper lymphocytes (T<sub>H</sub>). T<sub>H</sub> cells induce proliferation of both B and T lymphocytes, thus amplifying the immune response to the particular antigenic peptide that is displayed (Takahashi, *Microbiol. Immunol.*, 37:1-9, 1993).

Two distinct antigen processing pathways are associated with the two MHC classes. Intracellular antigens, synthesized inside of the cell, such as from viral or newly synthesized cellular proteins, for example, are processed and presented by Class I MHC. Exogenous antigens, taken up by the antigen-presenting cell (APC) from outside of the cell through endocytosis, are processed and presented by Class II MHC. After the antigenic material is proteolytically processed by the MHC-bearing cell, the resulting antigenic peptide forms a complex with the antigen binding groove of the MHC molecule through various noncovalent associations. The MHC-peptide complex on the cell surface is recognized by a specific T cell receptor on a cytotoxic or helper T cell.

The MHC of humans (also referred to as human leukocyte antigens (HLA)) on chromosome 6 has three loci, HLA-A, HLA-B and HLA-C, the first two of which have a large number of alleles encoding alloantigens. An adjacent region, known as HLA-D, is subdivided into HLA-DR, HLA-DQ and HLA-DP. The HLA region is now known as the human MHC region, and is equivalent to the H-2 region in mice. HLA-A, -



B and -C resemble mouse H-2K, -D, and -L and are the Class I MHC molecules. HLA-DP, -DQ and -DR resemble mouse I-A and I-E and are the Class II molecules. MHC glycoproteins of both classes have been isolated and characterized (*see Fundamental Immunology*, 2d Ed., W.E. Paul (ed.), Ravens Press, N.Y. (1989); and Roitt *et al.*,

5 *Immunology*, 2d Ed., Gower Medical Publishing, London (1989) , which are both incorporated herein by reference).

Human MHC Class I molecules consist of a polymorphic type I integral membrane glycoprotein heavy chain of about 46 kD, noncovalently associated with a 12 kD soluble subunit,  $\beta_2$ -microglobulin. The heavy chain consists of two distinct  
10 extracellular regions, the membrane distal, peptide binding region formed by the  $\alpha 1$  and  $\alpha 2$  domains, and the membrane proximal, CD8-binding region derived from the  $\alpha 3$  domain.  $\beta_2$ - microglobulin is a single, compact immunoglobulin-like domain that lacks a membrane anchor, and exists either associated with the class I heavy chain or free in plasma (Germain and Margulies, *Annu. Rev. Immunol.* 11:403-50, 1993).

15 Human MHC Class II is a heterodimeric integral membrane protein. Each dimer consists of one  $\alpha$  and one  $\beta$  chain in noncovalent association. The two chains are similar to each other, with the  $\alpha$  chain having a molecular weight of 32-34 kD and the  $\beta$  chain having a molecular weight of 29-32 kD. Both polypeptide chains contain N-linked oligosaccharide groups and have extracellular amino termini and intracellular  
20 carboxy termini.

The extracellular portions of the  $\alpha$  and  $\beta$  chain that comprise the class II molecule have been subdivided into two domains of about 90 amino acids each, called  $\alpha 1$ ,  $\alpha 2$ , and  $\beta 1$ ,  $\beta 2$ , respectively. The  $\alpha 2$  and  $\beta 2$  domains each contain a disulfide-linked loop. The peptide-binding region of the class II molecule is formed by the interaction of  
25 the  $\alpha 1$  and  $\beta 1$  domains. This interaction results in an open-ended, antigenic peptide-binding groove made up of two  $\alpha$  helices, and an eight-stranded  $\beta$ -pleated sheet platform.

The  $\alpha$  and  $\beta$  chains of Class II molecules are encoded by different MHC genes and are polymorphic (*see Addas et al., Cellular and Molecular Immunology*, 2d Ed., W.B. Saunders Co., New York (1994), which is incorporated by reference in its  
30 entirety). Within the present invention, a preferred  $\alpha$  chain is DRA\*0101 and a preferred  $\beta$  chain is DR $\beta$ 1\*1501.

## MHC Class II alleles

The soluble, fused heterodimer MHC class II:peptide complexes of the present invention can incorporate cDNA from any allele that predisposes or increases the likelihood of susceptibility to a specific autoimmune disease. Specific autoimmune diseases are correlated with specific MHC types. Specific haplotypes have been associated with many of the autoimmune diseases. For example, HLA-DR2<sup>+</sup> and HLA-DR3<sup>+</sup> individuals are at a higher risk than the general population to develop systemic lupus erythematosus (SLE) (Reinertsen *et al.*, *N. Engl. J. Med.* 299:515-18, 1970). Myasthenia gravis has been linked to HLA-D (Safwenberg *et al.*, *Tissue Antigens* 12:136-42, 1978). Susceptibility to rheumatoid arthritis is associated with HLA-D/DR in humans. Methods for identifying which alleles, and subsequently which MHC-encoded polypeptides, are associated with an autoimmune disease are known in the art. Exemplary alleles for IDDM include DR4, DQ8, DR3, DQ3.2.

## 15 Uses of soluble, fused MHC heterodimers

Soluble, fused MHC class II heterodimer:peptide complexes of the present invention can be used as antagonists to therapeutically block the binding of particular T cells and antigen-presenting cells. In addition, the molecules can induce anergy, or proliferative nonresponsiveness, and possibly apoptosis, in targeted T cells, both *in vivo* and *in vitro*. A soluble, fused MHC heterodimer:peptide molecule directed toward a desired autoimmune disease contains the antigenic peptide implicated for that autoimmune disease properly positioned in the binding groove of the MHC molecule, without need for solubilization of MHC or exogenous loading of an independently manufactured peptide.

Previous methods for producing desirable MHC Class II histocompatibility proteins have provided material that contains a mixture of antigenic peptides (Buus *et al.*, *Science* 242:1045-1047, 1988; and Rudensky *et al.*, *Nature* 353:622-627, 1991), which can be only partially loaded with a defined antigenic peptide (Watts and McConnel, *Pro. Natl. Acad. Sci. USA* 83:9660-64, 1986; and Ceppellini *et al.*, *Nature* 339:392-94, 1989). Various methods have been developed to produce heterodimers that do not present endogenous antigens (Stern and Wiley, *Cell* 68:465-77, 1992; Ljunggren *et al.*, *Nature* 346:476-80, 1990; and Schumacher *et al.*, *Cell* 62:563-67, 1990) that can be loaded with a

peptide of choice. WO 95/23814 and Kozono *et al.* have described production of soluble murine Class II molecules, I-E<sup>dk</sup> and I-A<sup>d</sup>, each with a peptide attached by a linker to the N terminus of the  $\beta$  chain. Ignatowicz *et al.* (*J. Immunol.* 154:38-62, 1995) have expressed membrane-bound I-A<sup>d</sup> with peptide attached. These methods incorporate the use of both membrane-bound heterodimer and soluble heterodimer.

The current invention offers the advantage of a soluble, fused MHC heterodimer made up of two or more MHC domains joined together via a flexible linkage, and onto which is tethered (via an additional flexible linkage) an antigenic peptide which is able to bind to the peptide binding groove presented by the soluble, fused MHC heterodimer. Such a complex provides an MHC molecule which is soluble and, because the components of the heterodimer and corresponding antigenic peptide are permanently linked into a single chain configuration, there is no need for complex heterodimer truncation or formation. These complexes eliminate inefficient and nonspecific peptide loading. Producing the claimed MHC:peptide complexes by recombinant methodology results in specific, high yield protein production, where the final product contains only the properly configured MHC:peptide complex of choice.

As used herein, a soluble heterodimer is one that does not contain membrane-associated MHC. The soluble MHC heterodimer of the present invention has never been membrane-associated. Further, the polypeptides contained within the MHC heterodimer do not contain an amino acid sequence that acts as a transmembrane domain or as a cytoplasmic domain.

The present invention therefore provides a soluble, fused MHC heterodimer which contains an antigenic peptide covalently attached to the amino terminal portion of an  $\alpha$  or  $\beta$  chain of MHC through a peptide linkage, and the C terminal of the linked  $\alpha$  or  $\beta$  chain may be attached to the N terminal portion of another  $\alpha$  or  $\beta$  chain, thereby creating a two, three, or four domain MHC molecule. The invention further provides a multimerization domain to provide a multimeric MHC class II heterodimer. The invention further provides novel linkers, and multimeric MHC class II heterodimers that are bound to different antigenic peptides.

The amino acid sequence of each of a number of Class I and Class II proteins are known, and the genes or cDNAs have been cloned. Thus, these nucleic acids can be used to express MHC polypeptides. If a desired MHC gene or cDNA is not

available, cloning methods known to those skilled in the art may be used to isolate the genes. One such method that can be used is to purify the desired MHC polypeptide, obtain a partial amino acid sequence, synthesize a nucleotide probe based on the amino acid sequence, and use the probe to identify clones that harbor the desired gene from a  
5 cDNA or genomic library.

### Linkers

Linkers of the current invention may be from about 1 to about 50 amino acids in length, depending on the molecular model of the MHC or MHC:peptide complex.  
10 In one embodiment, flexible linkers are made of repeating Gly residues separated by one or more Ser residues to permit a random, flexible motion. In the case of Class II MHC complexes this flexibility accommodates positioning of the  $\alpha$  and  $\beta$  segments to properly configure the binding groove, and also allows for maximum positioning of the peptide in the groove. In another embodiment, the linker comprises a CD4 binding site, as  
15 described below in the Example section (see also Table 1). In another embodiment, longer linkers between the chains contain flexible residues (e.g. alanine or glycine) and polar residues (e.g. serine and threonine). To inhibit the continuation of secondary structure across the linker, prolines can be added to bracket the linkers. These prolines are known to inhibit the formation of alpha helices and beta sheets. In another  
20 embodiment, flexible regions present in the human MHC and in the murine MHC could be used to make a linker by extending the region of interest and ligating the ends together. Finally, a combination of these types of linkers could also be used.

Linker position and length can be modeled based on the crystal structure of MHC Class II molecules (Brown *et al.*, *Nature* 364:33-39, 1993), where  $\alpha 1$  and  $\beta 1$  are  
25 assembled to form the peptide binding groove. Linkers joining segments of the  $\alpha$  and  $\beta$  chains together are based on the geometry of the region in the hypothetical binding site and the distance between the C terminus and the N terminus of the relevant segments. Molecular modeling based on the X-ray crystal structure of Class II MHC (Stern *et al.*, *Nature* 368:215-221, 1994) dictates the length of linkers joining antigenic peptide,  $\alpha$  chain  
30 segments and  $\beta$  chain segments. The recombinant portions of the molecules of the invention can also be directly linked, without additional amino acids linkers.

**Identification of autoantigens**

The invention also provides methods for preparing responder T-cell clones that proliferate when combined with a selected antigenic peptide presented by a stimulator cell. Such clones can be used to identify and map antigenic peptides associated with autoimmune disease. These peptides can then be incorporated into the soluble, fused MHC heterodimer:peptide complexes of the invention. The method provides isolation and enrichment of non-adherent, CD56<sup>-</sup>, CD8<sup>-</sup> T cells that are reactive with a selected antigenic peptide. These cells are herein referred to as responder cells.

Suitable responder cells can be isolated, for example, from peripheral blood mononuclear cells (PBMNC) obtained from patients prior to or after onset of an autoimmune disease of interest. For example, PBMNCs can be obtained from prediabetic and new onset diabetic patients. These patients can be pre-screened for specific HLA markers, such as DR3-DR4 or DQ3.2, which have the highest association with susceptibility to IDDM. From the collected PBMNCs, a portion is kept to serve as stimulator cells. From the remainder, the desired autoreactive responder cells are purified and isolated by two rounds of plating, to remove adherent cells from the population, followed by removal of monocytes and B cells with nylon wool. Enrichment for non-adherent CD4<sup>+</sup> T cells is completed by sequential plating of the cells onto plates coated with anti-CD8 and anti-CD56 antibodies.

The stimulator cells are pulsed or primed with whole GAD or an appropriate antigenic peptide. For example, stimulator cells from the PBMNCs of IDDM patients can be stimulated with antigenic GAD peptides then combined with PBMNCs or responder cells. After seven or 14 days, responder cell (T cell) clones are generated through limiting dilution and tested for antigen reactivity.

These responder cell (T cell) clones can then be used, for example, to map epitopes which bind to MHC and are recognized by a particular T cell. One such method uses overlapping peptide fragments of the autoantigen which are generated by tryptic digestion, or more preferably, overlapping peptides are synthesized using known peptide synthesis techniques. The peptide fragments are then tested for their ability to stimulate the responder T cell clones or lines (for example, Ota *et al.*, *Nature* 346:183-187, 1990).

Once such a peptide fragment has been identified, synthetic antigenic peptides can be specifically designed, for example, to enhance the binding affinity for

MHC and to out-compete any naturally processed peptides. Such synthetic peptides, when combined into a soluble, fused MHC heterodimer:peptide complex, would allow manipulation of the immune system *in vivo*, in order to tolerize or anergize disease-associated activated T cells, thereby ameliorating the autoimmune disease.

5 Dissecting the functional role of individual peptides and peptide clusters in the interaction of a peptide ligand with an MHC molecule, and also in subsequent T cell recognition and reactivity, is a difficult undertaking due to the degeneracy of peptide binding to the MHC. Changes in T cell recognition or in the ability of an altered peptide to associate with MHC can be used to establish that a particular amino acid or group of  
10 amino acids comprises part of an MHC or T cell determinant. The interactions of altered peptides can be further assessed by competition with the parental peptide for presentation to a T cell, or through development of direct peptide-MHC binding assays. Changes to a peptide that do not involve MHC binding could well affect T cell recognition. For example, in a peptide, specific MHC contact points might only occur within a central core  
15 of a few consecutive or individual amino acids, whereas those amino acids involved in T cell recognition may include a completely different subset of residues.

In a preferred method, residues that alter T cell recognition are determined by substituting amino acids for each position in the peptide in question, and by assessing whether such change in residues alters the peptide's ability to associate with MHC (Allen  
20 *et al.*, *Nature* 327:713-15, 1987; Sette *et al.*, *Nature* 328:395-99, 1987; O'Sullivan *et al.*, *J. Immunol.* 147:2663-69, 1991; Evavold *et al.*, *J. Immunol.* 148:347-53, 1992; Jorgensen *et al.*, *Annu. Rev. Immunol.* 10:835-73, 1992; Hammer *et al.*, *Cell* 74:197-203, 1993; Evavold *et al.*, *Immunol. Today* 14:602-9, 1993; Hammer *et al.*, *Proc. Natl. Acad. Sci. USA* 91:4456-60, 1994; and Reich *et al.*, *J. Immunol.* 154:2279-88, 1994). One method  
25 would involve generating a panel of altered peptides wherein individual or groups of amino acid residues are substituted with conservative, semi-conservative or non-conservative residues. A preferred variant of this method is an alanine scan (Ala scan) where a series of synthetic peptides are synthesized wherein each individual amino acid is substituted with L-alanine (L-Ala scan). Alanine is the amino acid of choice because it is  
30 found in all positions (buried and exposed), in secondary structure, it does not impose steric hindrances, or add additional hydrogen bonds or hydrophobic side chains. Alanine substitutions can be done independently or in clusters depending on the information

desired. Where the information pertains to specific residues involved in binding, each residue in the peptide under investigation can be converted to alanine and the binding affinity compared to the unsubstituted peptide. Additional structural and conformational information regarding each residue and the peptide as a whole can be gained, for

5 example, by synthesizing a series of analogs wherein each residue is substituted with a D-amino acid such as D-alanine (D-Ala scan) (Galantino *et al.*, in Smith, J. and Rivier, J. (eds.), *Peptides Chemistry and Biology (Proceedings of the Twelfth American Peptide Symposium)*, ESCOM, Leiden, 1992, pp. 404-05). Essential residues can be identified, and nonessential residues targeted for modification, deletion or replacement by other  
10 residues that may enhance a desired quality (Cunningham and Wells, *Science* 244:1081-1085, 1989; Cunningham and Wells, *Natl. Acad. Sci. USA*, 88:3407-3411, 1991; Ehrlich *et al.*, *J. Biol. Chem.* 267:11606-11, 1992; Zhang *et al.*, *Proc. Natl. Acad. Sci. USA* 90:4446-50, 1993; see also "Molecular Design and Modeling: Concepts and Applications Part A Proteins, Peptides, and Enzymes," *Methods in Enzymology*, Vol. 202, Langone  
15 (ed.), Academic Press, San Diego, CA, 1991).

Truncated peptides can be generated from the altered or unaltered peptides by synthesizing peptides wherein amino acid residues are truncated from the N- or C-terminus to determine the shortest active peptide, or between the N- and C-terminus to determine the shortest active sequence. Such peptides could be specifically developed to  
20 stimulate a response when joined to a particular MHC to form a peptide ligand to induce anergy in appropriate T cells *in vivo* or *in vitro*.

### **Analysis of MHC class II heterodimer:peptide complexes**

The physical and biological properties of the soluble, fused MHC  
25 heterodimer:peptide complexes may be assessed in a number of ways. Mass spectral analysis methods such as electrospray and Matrix-Assisted Laser Desorption/Ionization Time Of Flight mass spectrometry (MALDI TOF) analysis are routinely used in the art to provide such information as molecular weight and confirm disulfide bond formation. FACs analysis can be used to determine proper folding of the single chain complex.

30 An ELISA (Enzyme-linked Immunosorbent Assay) can be used to measure concentration and confirm correct folding of the soluble, fused MHC heterodimer:peptide complexes. This assay can be used with either whole cells;

solubilized MHC, removed from the cell surface; or free soluble, fused MHC heterodimer:peptide complexes of the current invention. In an exemplary ELISA, an antibody that detects the recombinant MHC haplotype is coated onto wells of a microtiter plate. In a preferred embodiment, the antibody is L243, a monoclonal antibody that  
5 recognizes only correctly folded HLA-DR MHC dimers. One of skill in the art will recognize that other MHC Class II-specific antibodies are known and available.

Alternatively, there are numerous routine techniques and methodologies in the field for producing antibodies (for example, Hurrell, J.G.R. (ed.), *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press Inc., Boca Raton, FL, 1982), if an

10 appropriate antibody for a particular haplotype does not exist. Anti-MHC Class II antibodies can also be used to purify Class II molecules through techniques such as affinity chromatography, or as a marker reagent to detect the presence of Class II molecules on cells or in solution. Such antibodies are also useful for Western analysis or immunoblotting, particularly of purified cell-secreted material. Polyclonal, affinity  
15 purified polyclonal, monoclonal and single chain antibodies are suitable for use in this regard. In addition, proteolytic and recombinant fragments and epitope binding domains can be used herein. Chimeric, humanized, veneered, CDR-replaced, reshaped or other recombinant whole or partial antibodies are also suitable.

In the ELISA format, bound MHC molecules can be detected using an  
20 antibody or other binding moiety capable of binding MHC molecules. This binding moiety or antibody may be tagged with a detectable label, or may be detected using a detectably labeled secondary antibody or binding reagent. Detectable labels or tags are known in the art, and include fluorescent, colorimetric and radiolabels, for instance.

Other assay strategies can incorporate specific T-cell receptors to screen  
25 for their corresponding MHC-peptide complexes, which can be done either *in vitro* or *in vivo*. For example, an *in vitro* anergy assay determines if non-responsiveness has been induced in the T cells being tested. Briefly, an MHC molecule containing antigenic peptide in the peptide binding groove can be mixed with responder cells, preferably peripheral blood mononuclear cells (PBMN) (a heterogeneous population including B and  
30 T lymphocytes, monocytes and dendritic cells), PBMNC lymphocytes, freshly isolated T lymphocytes, *in vivo* primed splenocytes, cultured T cells, or established T cell lines or



clones. Responder cells from mammals immunized with, or having a demonstrable cellular immune response to, the antigenic peptide are particularly preferred.

Subsequently, these responder cells are combined with stimulator cells (antigen presenting cells; APCs) that have been pulsed or primed with the same antigenic peptide. In a preferred embodiment, the stimulator cells are antigenic peptide-presenting cells, such as PBMNCs, PBMNCs that have been depleted of lymphocytes, appropriate antigenic peptide-presenting cell lines or clones (such as EBV-transformed B cells), EBV transformed autologous and non-autologous PMNCs, genetically engineered antigen presenting cells, such as mouse L cells or bare lymphocyte cells BLS-1, in particular, DRB1\*0401, DRB1\*0404 and DRB1\*0301 (Kovats et al, *J. Exp. Med.* 179:2017-22, 1994), or *in vivo* or *in vitro* primed or pulsed splenocytes. Stimulator cells from mammals immunized with, or having a demonstrable cellular immune response to, the antigenic peptide are particularly preferred. For certain assay formats, it is preferred to inhibit the proliferation of stimulator cells prior to mixing with responder cells. This inhibition may be achieved by exposure to gamma irradiation or to an anti-mitotic agent, such as mitomycin C, for instance. Appropriate negative controls are also included (nothing; syngeneic APC; experimental peptide; APC + Peptide; MHC:peptide complex; control peptide +/- APC). Further, to assure that non-responsiveness represents anergy, the proliferation assay may be set up in duplicate, +/- recombinant IL-2 since it has been demonstrated that IL-2, can rescue anergized cells.

After an approximately 72 hour incubation, the activation of responder cells in response to the stimulator cells is measured. In a preferred embodiment, responder cell activation is determined by measuring proliferation using <sup>3</sup>H-thymidine uptake (Crowley et al., *J. Immunol. Meth.* 133:55-66, 1990). Alternatively, responder cell activation can be measured by the production of cytokines, such as IL-2, or by determining the presence of responder cell-specific, and particularly T cell-specific, activation markers. Cytokine production can be assayed by testing the ability of the stimulator + responder cell culture supernatant to stimulate growth of cytokine-dependent cells. Responder cell- or T cell-specific activation markers may be detected using antibodies specific for such markers.

Preferably, the soluble, fused MHC heterodimer:peptide complex induces non-responsiveness (for example, anergy) in the antigenic peptide-reactive responder

cells. In addition to soluble, fused MHC heterodimer:peptide complex recognition, responder cell activation requires the involvement of co-receptors on the stimulator cell (the APC) that have been stimulated with co-stimulatory molecules. By blocking or eliminating stimulation of such co-receptors (for instance, by exposing responder cells to purified soluble, fused MHC heterodimer:peptide complex, by blocking with anti-receptor or anti-ligand antibodies, or by "knocking out" the gene(s) encoding such receptors), responder cells can be rendered non-responsive to antigen or to soluble, fused MHC heterodimer:peptide complex.

In a preferred embodiment, responder cells are obtained from a source manifesting an autoimmune disease or syndrome. Alternatively, autoantigen-reactive T cell-clones or lines are preferred responder cells. In another preferred embodiment, stimulator cells are obtained from a source manifesting an autoimmune disease or syndrome. Alternatively, APC cell lines or clones that are able to appropriately process and/or present autoantigen to responder cells are preferred stimulator cells. In a particularly preferred embodiment, responder and stimulator cells are obtained from a source with diabetes or multiple sclerosis.

At this point, the responder T cells can be selectively amplified and/or stimulated, thereby producing a subset of T cells that are specific for the antigenic peptide. For instance, antigenic peptide-reactive responder cells may be selected by flow cytometry, and particularly by fluorescence activated cell sorting. This subset of responder cells can be maintained by repetitive stimulation with APCs presenting the same antigenic peptide. Alternatively, responder cell clones or lines can be established from this responder cell subset. Further, this subset of responder cells can be used to map epitopes of the antigenic peptide and the protein from which it is derived.

Other methods to assess the biological activity of the soluble, fused MHC heterodimer:peptide complexes are known in the art and can be used herein, such as using a microphysiometer, to measure production of acidic metabolites in T cells following interaction with antigenic peptide. Other assay methods include competition assays, comparing soluble, fused MHC heterodimer:complex response with that to the normal antigen. Also measurement production of such indicators as cytokines or  $\gamma$  interferon can provide an indication of complex response.

### Animal models of autoimmune disease

Similar assays and methods can be developed for and used in animal models. For instance, the therapeutic effect of a pharmaceutical composition of the single chain molecule or multimer or a polynucleotide encoding the single chain molecule or multimer can be tested *in vivo* in a number of animal models of HLA-DR-associated autoimmune disease. These diseases include, but are not limited to, insulin-dependent diabetes mellitus, multiple sclerosis, myasthenia gravis, pernicious anemia, rheumatoid arthritis, and systemic lupus erythematosus.

For example, NOD mice are a spontaneous model of IDDM. Treatment with the pharmaceutical compositions prior to or after onset of disease can be monitored by assay of urine glucose levels in the NOD mouse, as well as by *in vitro* T cell proliferation assays to assess reactivity to known autoantigens (*see, e.g., Kaufman et al., Nature* 366:69-72 (1993)) for example). Alternatively, induced models of autoimmune disease, such as EAE, can be treated with pharmaceutical composition. Treatment in a preventive or intervention mode can be followed by monitoring the clinical symptoms of EAE.

Following is a description of several other animal models of HLA-DR-associated autoimmune disease which can be used to assay *in vivo* effects of the peptide. It will be obvious to one of skill in the art that other suitable animal models for autoimmune diseases can be utilized in a similar manner.

#### Systemic Lupus Erythematosus (SLE)

F<sub>1</sub> hybrids of autoimmune New Zealand black (NZB) mice and the phenotypically normal New Zealand White (NZW) mouse strain develop severe systemic autoimmune disease, more fulminant than that found in the parental NZB strain. These mice manifest several immune abnormalities, including antibodies to nuclear antigens and subsequent development of a fatal, immune complex-mediated glomerulonephritis with female predominance, remarkably similar to SLE in humans (Knight *et al., J. Exp. Med.* 147:1653 (1978)), which is incorporated hereby by reference.

In both the human and murine forms of the disease, a strong association with MHC gene products has been reported. HLA-DR2 and HLA-DR3 individuals are at a higher risk than the general population to develop SLE (Reinertsen *et al., N. Engl. J.*

*Med.* 299:515 (1970)), while in NZB/W F<sub>1</sub> mice (H-2<sup>d/u</sup>), a gene linked to the h-2<sup>u</sup> haplotype derived from the NZW parent contributes to the development of the lupus-like nephritis.

The effect of the invention can be measured by survival rates and by the progress of development of the symptoms, such as proteinuria and appearance of anti-DNA antibodies.

Proteinuria can be measured by any method known to those of skill in the art, *e.g.* colorimetrically by the use of Uristix (Miles Laboratories, Inc., Elkhart, IN), giving an approximation of proteinuria as follows: trace, 10 mg/dl; 1+, 30 mg/dl; 100 mg/dl; 3+, 300 mg/dl; and 4+, 1000 mg/dl.

-- The presence of anti-DNA specific antibodies in NZB/W F<sub>1</sub> mice can be determined by using a modification of a linked immunosorbent assay (ELISA) described by Zouali *et al.*, *J. Immunol. Methods* 90:105 (1986)) which is incorporated herein by reference.

#### Myasthenia Gravis (MG)

Myasthenia gravis is one of several human autoimmune diseases linked to HLA-D (Safenberg, *et al.*, *Tissue Antigens* 12:136 (1978); McDevitt *et al.*, *Arth. Rheum.* 20:59 (1977)) which are incorporated herein by reference. In MG antibodies to the acetyl choline receptors (AcChoR) impair neuromuscular transmission by mediating loss of AcChoR in the postsynaptic membrane.

SJL/J female mice are a model system for human MG. In these animals, experimental autoimmune myasthenia gravis (EAMG) can be induced by immunizing the mice with soluble AcChoR protein from another species. Susceptibility to EAMG is linked in part to the MHC and has been mapped to the region within H-2 (Christadoss *et al.*, *J. Immunol.* 123:2540 (1979)).

AcChoR protein can purified from *Torpedo californica* and assayed according to the method of Waldor *et al.*, *Proc. Natl. Acad. Sci. USA* 80:2713 (1983), incorporated by reference. For example, emulsified AcChoR, 15 µg in complete Freund adjuvant, is injected intradermally among six sites on the back, the hind foot pads, and the base of the tail. Animals are reimmunized with this same regimen 4 weeks later.

Evaluation can be made by measurement of anti-AcChoR antibodies by any method known to those of skill in the art, *e.g.*, a microtiter ELISA assay as described in Waldor *et al.*, *supra*. In an exemplary assay, the standard reagent volume is 50  $\mu$ l per well. Reagents are usually incubated in the wells for 2 hr at RT. Five  $\mu$ g of AcChoR  
 5 diluted in bicarbonate buffer, pH 9.6, is added to each well. After incubation with AcChoR, the plates are rinsed four times with a wash solution consisting of phosphate-buffer saline containing 0.05% Tween and 0.05%  $\text{NaN}_3$ . Mouse sera are diluted in 0.01M PBS (pH 7.2), 1.5 mfr  $\text{MgCl}_2$ , 2.0 mM 2-mercaptoethanol, 0.05% Tween-80, 0.05%  $\text{NaN}_3$  (p-Tween buffer) and incubated on the plate. After the plate is washed, beta-  
 10 galactosidase-conjugated sheep anti-mouse antibody diluted in P-Tween buffer is added to each well. After a final washing, the enzyme substrate, p-nitrophenylgalctopyranoside is added to the plate, and the degree of substrate catalysis is determined from the absorbance at 405 nm after 1 hr.

Anti-AcChoR antibodies are expected to be present in the mice immunized  
 15 with AcChoR as compared to nonimmunized mice. Treatment with complex is expected to significantly reduce the titer of anti-AcChoR antibodies in the immunized mice.

The effect of treatment with the invention on clinical EAMG can also be assessed by any method known to those of skill in the art. Myasthenia symptoms include a characteristic hunched posture with drooping of the head and neck, exaggerated arching  
 20 of the back, splayed limbs, abnormal walking, and difficulty in righting. Mild symptoms are present after a standard stress test, and should be ameliorated by administration of complex.

#### Rheumatoid Arthritis (RA)

25 In humans, susceptibility to rheumatoid arthritis is associated with HLA D/DR. The immune response in mice to native type II collagen has been used to establish an experimental model for arthritis with a number of histological and pathological features resembling human RA. Susceptibility to collagen-induced arthritis (CIA) in mice has been mapped to the H-2 I region, particularly the I-A subregion (Huse *et al.*, *Fed.*  
 30 *Proc.* 43:1820 (1984)).

Mice from a susceptible strain, DEA-1 can be caused to have CIA by treatment of the mice with native type II collagen, using the technique described in Wooley *et al.*, *J. Immunol.* 134:2366 (1985), incorporated herein by reference.

In another model adjuvant arthritis in rats is an experimental model for human arthritis, and a prototype of autoimmune arthritis triggered by bacterial antigens (Holoschitz *et al.*, *Prospects of Immunology* (1986); Pearson, *Arthritis Rheum.* 7:80 (1964)). The disease is the result of a cell-mediated immune response, as evidenced by its transmissibility by a clone of T cells which were reactive against the adjuvant (MT); the target self-antigen in the disease, based upon studies with the same cloned cells, appears to be part(s) of a proteoglycan molecule of cartilage.

Adjuvant disease in rats is produced as described by Pearson *supra*, *i.e.*, by a single injection of Freund's adjuvant (killed tubercle bacilli or chemical fractions of it, mineral oil, and an emulsifying agent) given into several depot sites, preferably intracutaneously or into a paw or the base of the tail. The adjuvant is given in the absence of other antigens.

The effect of the invention treatment on manifestations of the disease can be monitored by any method known to those of skill in the art. These manifestations are histopathological, and include an acute and subacute synovitis with proliferation of synovial lining cells, predominantly a mononuclear infiltration of the articular and particular tissues, the invasion of bone and articular cartilage by connective tissue pannus, and periosteal new bone formation, especially adjacent to affected joints. In severe or chronic cases, destructive changes occur, as do fibrous or bony ankylosis. These histopathological symptoms are expected to appear in control animals at about 12 days after sensitization to the Freund's adjuvant.

### Insulin Dependent Diabetes Mellitus (IDDM)

IDDM is observed as a consequence of the selective destruction of insulin-secreting cells within the Islets of Langerhans of the pancreas. Involvement of the immune system in this disease is suggested by morphologic evidence of early infiltration of the Islets by mononuclear cells, by the detection of anti-islet cell antibodies, by the high frequency of HLA-DR3 and -DR4 alleles in IDDM populations, and by clinical associations between IDDM and various autoimmune diseases. An animal model for

spontaneous IDDM and thyroiditis has been developed in the BB rat. As in humans, the rat disease is controlled in part by the genes encoding the MHC antigens, is characterized by islet infiltration, and is associated with the presence of anti-islet antibodies. The I-E equivalent class II MHC antigens appear to be involved in manifestation of the autoimmune diseases in the BB rat. Biotard *et al.*, *Proc. Natl. Acad. Sci. USA* 82:6627 (1985).

In morphologic evaluation, insulinitis is characterized by the presence of mononuclear inflammatory cells within the islets. Thyroiditis is characterized by focal interstitial lymphocytic infiltrate within the thyroid gland, as a minimum criterion. Most severe cases show diffuse extensive lymphocytic infiltrates, disruption of acini, fibrosis, and focal Hurthle cell change. See Biotard *et al. supra*.

Treatment of the BB rats with the invention is expected to ameliorate or prevent the manifestation of the clinical and morphological symptoms associated with IDDM and thyroiditis.

In another model, the NOD mouse strain (H-2K<sup>d</sup> D<sup>b</sup>) is a murine model for autoimmune IDDM. The disease in these animals is characterized by anti-islet cell antibodies, severe insulinitis, and evidence for autoimmune destruction of the beta-cells (Kanazawa, *et al.*, *Diabetologia* 27:113 (1984)). The disease can be passively transferred with lymphocytes and prevented by treatment with cyclosporin-A (Ikehara *et al.*, *Proc. Natl. Acad. Sci. USA* 82:7743 (1985)); Mori *et al.*, *Diabetologia* 29:244 (1986). Untreated animals develop profound glucose intolerance and ketosis and succumb within weeks of the onset of the disease. Seventy to ninety percent of female and 20-30% of male animals develop diabetes within the first six months of life. Breeding studies have defined at least two genetic loci responsible for disease susceptibility, one of which maps to the MHC. Characterization of NOD Class II antigens at both the serologic and molecular level suggest that the susceptibility to autoimmune disease is linked to I-A<sub>B</sub> (Acha-Orbea and McDevitt, *Proc. Natl. Acad. Sci. USA* 84:235 (1987)).

Treatment of Female NOD mice with complex is expected to lengthen the time before the onset of diabetes and/or to ameliorate or prevent the disease.

### Experimental Allergic Encephalomyelitis (EAE)

Experimental allergic encephalomyelitis (EAE) is an induced autoimmune disease of the central nervous system which is a model for multiple sclerosis (MS). The disease can be induced in many species, including mice and rats.

5           The disease is characterized by the acute onset of paralysis. Perivascular infiltration by mononuclear cells in the CNS is observed in both mice and rats. Methods of inducing the disease, as well as symptomology, are reviewed in Aranson, *The Autoimmune Diseases* (Rose and Mackay, eds., 1985), and in Acha-Orbea *et al.*, *Ann. Rev. Imm.* 7:377-405 (1989).

10           One of the genes mediating susceptibility is localized in the MHC class II region (Moore *et al.*, *J. Immunol.* 124:1815-1820 (1980)). The best analyzed encephalitogenic protein is myelin basic protein (MBP), but other encephalitogenic antigens are found in the brain. The immunogenic epitopes have been mapped (*see*, Acha-Orbea *et al.*, *supra.*). In the PL mouse strains (H-2<sup>u</sup>) two encephalitogenic peptides in  
15 MBP have been characterized: MBP peptide p35-47 (MBP 35-47), and acetylated NSF p1-9 (MBP 1-9).

          The effect of the invention on ameliorating disease symptoms in individuals in which EAE has been induced can be measured by survival rates, and by the progress of the development of symptoms.

### **Methods of making the complexes of the invention**

          Expression systems suitable for production of appropriate soluble, fused MHC heterodimer:peptide complexes are available and known in the art. Various prokaryotic, fungal, and eukaryotic host cells are suitable for expression of soluble, fused  
25 MHC heterodimer:peptide complexes.

          Prokaryotes that are useful as host cells, according to the present invention, most frequently are represented by various strains of *Escherichia coli*. However, other microbial strains can also be used, such as bacilli, for example *Bacillus subtilis*, various species of *Pseudomonas*, or other bacterial strains.

30           According to the invention, the soluble, fused MHC heterodimer:peptide complexes are expressed from recombinantly engineered nucleotide sequences that encode the soluble, fused MHC heterodimer:peptide polypeptides by operably linking the



engineered nucleic acid coding sequence to signals that direct gene expression in prokaryotes. A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For instance, a promoter or enhancer is operably linked to a coding sequence if it effects the transcription of the sequence.

- 5 Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in reading frame.

The genes encoding the soluble, fused MHC heterodimer:peptide complexes may be inserted into an "expression vector," "cloning vector," or "vector," terms which are used interchangeably herein and usually refer to plasmids or other  
10 nucleic acid molecules that are able to replicate in a chosen host cell. Expression vectors may replicate autonomously, or they can replicate by being inserted into the genome of the host cell, by methods well known in the art. Vectors that replicate autonomously will have an origin of replication or autonomous replicating sequence (ARS) that is functional  
15 in the chosen host cell(s).

Plasmid vectors that contain replication sites and control sequences derived from a species compatible with the chosen host are used. For example, *E. coli* is typically transformed using derivatives of pBR322, a plasmid derived from *E. coli* species by Bolivar *et al.*, *Gene* 2:95-113, 1977. Often, it is desirable for a vector to be usable in  
20 more than one host cell, e.g., in *E. coli* for cloning and construction, and in a *Bacillus* cell for expression.

The expression vectors typically contain a transcription unit or expression cassette that contains all the elements required for the expression of the DNA encoding the MHC molecule in the host cells. A typical expression cassette contains a promoter  
25 operably linked to the DNA sequence encoding a soluble, fused MHC heterodimer:peptide complex and a ribosome binding site. The promoter is preferably positioned about the same distance from the heterologous transcription start site as it is from the transcription start site in its natural setting. As is known in the art, however, some variation in this distance can be accommodated without loss of promoter function.

30 In addition to a promoter sequence, the expression cassette can also contain a transcription termination region downstream of the structural gene to provide for efficient

termination. The termination region may be obtained from the same gene as the promoter sequence or may be obtained from a different gene.

Commonly used prokaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with an operator, along with  
 5 ribosome binding site sequences, include such commonly used promoters as the beta-lactamase (penicillinase) and lactose (lac) promoter systems (Change *et al.*, *Nature* 198:1056, 1977) and the tryptophan (trp) promoter system (Goeddel *et al.*, *Nucleic Acids Res.* 8:4057-74, 1980) and the lambda-derived P<sub>L</sub> promoter and N-gene ribosome binding site (Shimatake *et al.*, *Nature* 292:128-32, 1981). Any available promoter system that  
 10 functions in prokaryotes can be used.

Either constitutive or-regulated promoters can be used in the present invention. Regulated promoters can be advantageous because the host cells can be grown to high densities before expression of the soluble, fused MHC heterodimer:peptide complexes is induced. High level expression of heterologous proteins slows cell growth  
 15 in some situations. Regulated promoters especially suitable for use in *E. coli* include the bacteriophage lambda P<sub>L</sub> promoter, the hybrid *trp-lac* promoter (Amann *et al.*, *Gene* 25:167-78 1983; and the bacteriophage T7 promoter.

For expression of soluble, fused MHC heterodimer:peptide complexes in prokaryotic cells other than *E. coli*, a promoter that functions in the particular prokaryotic  
 20 species is required. Such promoters can be obtained from genes that have been cloned from the species, or heterologous promoters can be used. For example, the hybrid *trp-lac* promoter functions in *Bacillus* in addition to *E. coli*.

A ribosome binding site (RBS) is also necessary for expression of soluble, fused MHC heterodimer:peptide complexes in prokaryotes. An RBS in *E. coli*, for  
 25 example, consists of a nucleotide sequence 3-9 nucleotides in length located 3-11 nucleotides upstream of the initiation codon (Shine and Dalgarno, *Nature*, 254:34-40, 1975; Steitz, In Biological regulation and development: *Gene expression* (ed. R.F. Goldberger), vol. 1, p. 349, 1979, Plenum Publishing, NY).

Translational coupling may be used to enhance expression. The strategy  
 30 uses a short upstream open reading frame derived from a highly expressed gene native to the translational system, which is placed downstream of the promoter, and a ribosome binding site followed after a few amino acid codons by a termination codon. Just prior to

the termination codon is a second ribosome binding site, and following the termination codon is a start codon for the initiation of translation. The system dissolves secondary structure in the RNA, allowing for the efficient initiation of translation. See Squires, et. al., *J. Biol. Chem.* 263:16297-16302, 1988.

5                   The soluble, fused MHC heterodimer:peptide complexes can be expressed intracellularly, or can be secreted from the cell. Intracellular expression often results in high yields. However, some of the protein may be in the form of insoluble inclusion bodies. Although some of the intracellularly produced MHC polypeptides of the present invention may be active upon being harvested following cell lysis, the amount of soluble, active MHC polypeptide may be increased by performing refolding procedures using methods known to those of skill in the art (see, e.g., Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual Second Edition*, Cold Spring Harbor, NY, 1989.; Marston *et al.*, *Bio/Technology* 2:800-804, 1985; Schoner *et al.*, *Bio/Technology* 3:151-54, 1985). In one embodiment, for purification and refolding the cell pellet is lysed and refolded in urea-  
10 borate-DTT buffer followed by urea-borate buffer and reverse phase HPLC purification using either silica gel based Vydac (Hewlett Packard, Wilmington, DE) or polymer based Poros-R2 (PerSeptive Biosystems) resins, with bead size varying based on the scale of the culture and is described in further detail below. In one embodiment, e.g., for large scale refolding, the sample can be ultrafiltered into a urea-borate buffer to which is then added  
15 0.2  $\mu$ M to 1 mM copper sulfate, preferably 0.2 to 20  $\mu$ M, after which folding occurs immediately.

More than one MHC:peptide complex may be expressed in a single prokaryotic cell by placing multiple transcriptional cassettes in a single expression vector, or by utilizing different selectable markers for each of the expression vectors which are  
25 employed in the cloning strategy.

A second approach for expressing the MHC:peptide complexes of the invention is to cause the polypeptides to be secreted from the cell, either into the periplasm or into the extracellular medium. The DNA sequence encoding the MHC polypeptide is linked to a cleavable signal peptide sequence. The signal sequence directs  
30 translocation of the MHC:peptide complex through the cell membrane. An example of a suitable vector for use in *E. coli* that contains a promoter-signal sequence unit is pTA1S29, which has the *E. coli* *phoA* promoter and signal sequence see, e.g., Sambrook

*et al., supra*; Oka *et al.*, *Proc. Natl. Acad. Sci. USA* 82:7212-16, 1985; Talmadge *et al.*, *Proc. Natl. Acad. Sci. USA* 77:39892, 1980; Takahara *et al.*, *J. Biol. Chem.* 260: 2670-74, 1985). Once again, multiple polypeptides can be expressed in a single cell for periplasmic association.

5           The MHC:peptide complexes of the invention can also be produced as fusion proteins. This approach often results in high yields, because normal prokaryotic control sequences direct transcription and translation. In *E. coli*, lacZ fusions are often used to express heterologous proteins. Suitable vectors are readily available, such as the pUR, pEX, and pMR100 series (*see, e.g.*, Sambrook *et al., supra*). For certain  
10 applications, it may be desirable to cleave the non-MHC amino acids from the fusion protein after purification. This can be accomplished by any of several methods known in the art, including cleavage by cyanogen bromide, a protease, or by Factor X, (*see, e.g.* Sambrook *et al., supra.*; Goeddel *et al.*, *Proc. Natl. Acad. Sci. USA* 76:106-10, 1979; Nagai *et al.*, *Nature* 309:810-12, 1984; Sung *et al.*, *Proc. Natl. Acad. Sci. USA* 83:561-65,  
15 1986). Cleavage sites can be engineered into the gene for the fusion protein at the desired point of cleavage.

Foreign genes, such as soluble, fused MHC heterodimer:peptide complexes, can be expressed in *E. coli* as fusions with binding partners, such as glutathione-S-transferase (GST), maltose binding protein, or thioredoxin. These binding  
20 partners are highly translated and can be used to overcome inefficient initiation of translation of eukaryotic messages in *E. coli*. Fusion to such binding partner can result in high-level expression, and the binding partner is easily purified and then excised from the protein of interest. Such expression systems are available from numerous sources, such as Invitrogen Inc. (San Diego, CA) and Pharmacia LKB Biotechnology Inc.  
25 (Piscataway, NJ).

A method for obtaining recombinant proteins from *E. coli* which maintains the integrity of their N-termini has been described by Miller *et al.* *Biotechnology* 7:698-704 (1989). In this system, the gene of interest is produced as a C-terminal fusion to the first 76 residues of the yeast ubiquitin gene containing a peptidase cleavage site.  
30 Cleavage at the junction of the two moieties results in production of a protein having an intact authentic N-terminal residue.

The vectors containing the nucleic acids that code for the soluble, fused MHC heterodimer:peptide complexes are transformed into prokaryotic host cells for expression. "Transformation" refers to the introduction of vectors containing the nucleic acids of interest directly into host cells by well known methods. The particular procedure used to introduce the genetic material into the host cell for expression of the soluble, fused MHC heterodimer:peptide complex is not particularly critical. Any of the well known procedures for introducing foreign nucleotide sequences into host cells may be used. It is only necessary that the, particular host cell utilized be capable of expressing the gene.

Transformation methods, which vary depending on the type of the prokaryotic host cell, include electroporation; transfection employing calcium chloride, rubidium chloride calcium phosphate, or other substances; microprojectile bombardment; infection (where the vector is an infectious agent); and other methods. See, generally, Sambrook *et al., supra*, and Ausubel *et al., (eds.) Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987. Reference to cells into which the nucleic acids described above have been introduced is meant to also include the progeny of such cells. Transformed prokaryotic cells that contain expression vectors for soluble, fused MHC heterodimer:peptide complexes are also included in the invention.

After standard transfection or transformation methods are used to produce prokaryotic cell lines that express large quantities of the soluble, fused MHC heterodimer:peptide complex polypeptide, the polypeptide is then purified using standard techniques. See, e.g., Colley *et al., J. Chem.* 64:17619-22, 1989; and *Methods in Enzymology*, "Guide to Protein Purification", Deutscher, ed., Vol. 182 (1990). The recombinant cells are grown and the soluble, fused MHC heterodimer:peptide complex is expressed. The purification protocol will depend upon whether the soluble, fused MHC heterodimer:peptide complex is expressed intracellularly, into the periplasm, or secreted from the cell. For intracellular expression, the cells are harvested, lysed, and the polypeptide is recovered from the cell lysate (Sambrook *et al., supra*). Periplasmic MHC polypeptide is released from the periplasm by standard techniques (Sambrook *et al., supra*). If the MHC polypeptide is secreted from the cells, the culture medium is harvested for purification of the secreted protein. The medium is typically clarified by centrifugation or filtration to remove cells and cell debris.

The MHC polypeptides can be concentrated by adsorption to any suitable resin (such as, for example, CDP-Sepharose, Asialoprothrombin-Sepharose 4B, or Q Sepharose), or by use of ammonium sulfate fractionation, polyethylene glycol precipitation, or by ultrafiltration. Other means known in the art may be equally suitable.

Further purification of the MHC polypeptides can be accomplished by standard techniques, for example, affinity chromatography, ion exchange chromatography, sizing chromatography, reverse phase HPLC, or other protein purification techniques used to obtain homogeneity. The purified proteins are then used to produce pharmaceutical compositions.

For secretion of a polypeptide or protein of interest, recombinant nucleic acid constructs of the invention may include sequences that encode signal sequences or other sequences that direct secretion. Secretory signal sequences, also called leader sequences, prepro sequences and/or pre sequences, are amino acid sequences that play a role in secretion of mature polypeptides or proteins from a cell. Such sequences are characterized by a core of hydrophobic amino acids and are typically (but not exclusively) found at the amino termini of newly synthesized proteins. The secretory signal sequence may be that of the protein of interest, or may be derived from another secreted protein (e.g., t-PA, a preferred mammalian secretory leader) or synthesized de novo. The secretory signal sequence is joined to the DNA sequence encoding a protein of the present invention in the correct reading frame. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain signal sequences may be positioned elsewhere in the DNA sequence of interest (see, e.g., Welch *et al.*, U.S. Patent No. 5,037,743; Holland *et al.*, U.S. Patent No. 5,143,830). Very often the secretory peptide is cleaved from the mature protein during secretion. Such secretory peptides contain processing sites that allow cleavage of the secretory peptide from the mature protein as it passes through the secretory pathway. An example of such a processing site is a dibasic cleavage site, such as that recognized by the *Saccharomyces cerevisiae* KEX2 gene or a Lys-Arg processing site. Processing sites may be encoded within the secretory peptide or may be added to the peptide by, for example, *in vitro* mutagenesis.

Secretory signals include the  $\alpha$  factor signal sequence (prepro sequence: Kurjan and Herskowitz, *Cell* 3:933-943, 1982; Kurjan *et al.*, U.S. Patent No. 4,546,082;

Brake, EP 116, 201), the *PHO5* signal sequence (Beck *et al.*, WO 86/00637), the BAR1 secretory signal sequence (MacKay *et al.*, U.S. Patent No. 4,613,572; MacKay, WO 87/002670), the *SUC2* signal sequence (Carlsen *et al.*, *Molecular and Cellular Biology* 3: 439-447, 1983), the a-1-antitrypsin signal sequence (Kurachi *et al.*, *Proc. Natl. Acad. Sci. USA* 78: 6826-6830, 1981), the a-2 plasmin inhibitor signal sequence (Tone *et al.*, *J. Biochem. (Tokyo)* 102: 1033-1042, 1987) and the tissue plasminogen activator signal sequence (Pennica *et al.*, *Nature* 301: 214-221, 1983). Alternately, a secretory signal sequence may be synthesized according to the rules established, for example, by von Heinje (*European Journal of Biochemistry* 133: 17-21, 1983; *Journal of Molecular Biology* 184: 99-105, 1985; *Nucleic Acids Research* 14: 4683-4690; 1986). Another signal sequence is the synthetic signal LaC212 spx (1-47). ERLE described in WO 90/10075.

Secretory signal sequences may be used singly or may be combined. For example, a first secretory signal sequence may be used in combination with a sequence encoding the third domain of barrier (described in U.S. Patent No. 5,037,243, which is incorporated by reference herein in its entirety). The third domain of barrier may be positioned in proper reading frame 3' of the DNA segment of interest or 5' to the DNA segment and in proper reading frame with both the secretory signal sequence and a DNA segment of interest.

The choice of suitable promoters, terminators and secretory signals for all expression systems, is well within the level of ordinary skill in the art. Methods for expressing cloned genes in *Saccharomyces cerevisiae* are generally known in the art (see, "Gene Expression Technology," *Methods in Enzymology*, Vol. 185, Goeddel (ed.), Academic Press, San Diego, CA, 1990 and "Guide to Yeast Genetics and Molecular Biology," *Methods in Enzymology*, Guthrie and Fink (eds.), Academic Press, San Diego, CA, 1991; which are incorporated herein by reference). Proteins of the present invention can also be expressed in filamentous fungi, for example, strains of the fungi *Aspergillus* (McKnight *et al.*, U.S. Patent No. 4,935,349, which is incorporated herein by reference). Expression of cloned genes in cultured mammalian cells and in *E. coli*, for example, is discussed in detail in Sambrook *et al.* (*Molecular Cloning: A Laboratory Manual. Second Edition*, Cold Spring Harbor, NY, 1989; which is incorporated herein by reference). As would be evident to one skilled in the art, one could express the proteins

of the instant invention in other host cells such as avian, insect and plant cells using regulatory sequences, vectors and methods well established in the literature.

In yeast, suitable yeast vectors for use in the present invention include YRp7 (Struhl *et al.*, *Proc. Natl. Acad. Sci. USA* 76:1035-1039, 1978), YEpl3 (Broach *et al.*, *Gene* 8: 121-133, 1979), POT vectors (Kawasaki *et al.*, U.S. Patent No. 4,931,373, 5 which is incorporated by reference herein), pJDB249 and pJDB219 (Beggs, *Nature* 275:104-108, 1978) and derivatives thereof. Preferred promoters for use in yeast include promoters from yeast glycolytic genes (Hitzeman *et al.*, *J. Biol. Chem.* 255: 12073-12080, 1980; Alber and Kawasaki, *J. Mol. Appl. Genet.* 1: 419-434, 1982; Kawasaki, U.S. Patent 10 No. 4,599,311) or alcohol dehydrogenase genes (Young *et al.*, in *Genetic Engineering of Microorganisms for Chemicals*, Hollaender *et al.*, (eds.), p: 355, Plenum, New York, 1982; Ammerer, *Meth. Enzymol.* 101: 192-201, 1983). Other promoters are the TPI1 promoter (Kawasaki, U.S. Patent No. 4,599,311, 1986) and the ADH2-4<sup>c</sup> promoter (Russell *et al.*, *Nature* 304: 652-654, 1983; Irani and Kilgore, U.S. Patent Application 15 Serial No. 07/784,653, CA 1,304,020 and EP 284 044, which are incorporated herein by reference). The expression units may also include a transcriptional terminator such as the TPI1 terminator (Alber and Kawasaki, *ibid.*).

Yeast cells, particularly cells of the genus *Pichia* or *Saccharomyces*, are a preferred host for use in producing compound of the current invention. Methods for 20 transforming yeast cells with exogenous DNA and producing recombinant proteins therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki *et al.*, U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch *et al.*, U.S. Patent No. 5,037,743; and Murray *et al.*, U.S. Patent No. 4,845,075, which are incorporated herein by reference. Transformed cells are selected by phenotype 25 determined by a selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in yeast is the POT1 vector system disclosed by Kawasaki *et al.* (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. A preferred secretory signal sequence for use in yeast is that of the *S. cerevisiae* MF $\alpha$ 1 gene 30 (Brake, *ibid.*; Kurjan *et al.*, U.S. Patent No. 4,546,082). Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman *et al.*, U.S. Patent No. 4,615,974; and



Bitter, U.S. Patent No. 4,977,092, which are incorporated herein by reference) and alcohol dehydrogenase genes. See also U.S. Patent Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454, which are incorporated herein by reference. Transformation systems for other yeasts, including *Hansenula polymorpha*, *Schizosaccharomyces pombe*,

5 *Kluyveromyces lactis*, *Kluyveromyces fragilis*, *Ustilago maydis*, *Pichia pastoris*, *Pichia methanolica*, *Pichia guilliermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson *et al.*, *J. Gen. Microbiol.* 132:3459-65, 1986; Cregg, U.S. Patent No. 4,882,279; and Stroman *et al.*, U.S. Patent No. 4,879,231.

Other fungal cells are also suitable as host cells. For example, *Aspergillus*  
10 cells may be utilized according to the methods of McKnight *et al.*, U.S. Patent No. 4,935,349, which is incorporated herein by reference. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino *et al.*, U.S. Patent No. 5,162,228, which is incorporated herein by reference. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533, which is incorporated herein by  
15 reference.

Host cells containing DNA constructs of the present invention are then cultured to produce the heterologous proteins. The cells are cultured according to standard methods in a culture medium containing nutrients required for growth of the particular host cells. A variety of suitable media are known in the art and generally  
20 include a carbon source, a nitrogen source, essential amino acids, vitamins, minerals and growth factors. The growth medium will generally select for cells containing the DNA construct by, for example, drug selection or deficiency in an essential nutrient which is complemented by a selectable marker on the DNA construct or co-transfected with the DNA construct.

25 Yeast cells, for example, are preferably cultured in a chemically defined medium, comprising a non-amino acid nitrogen source, inorganic salts, vitamins and essential amino acid supplements. The pH of the medium is preferably maintained at a pH greater than 2 and less than 8, preferably at pH 6.5. Methods for maintaining a stable pH include buffering and constant pH control, preferably through the addition of sodium  
30 hydroxide. Preferred buffering agents include succinic acid and Bis-Tris (Sigma Chemical Co., St. Louis, MO). Yeast cells having a defect in a gene required for asparagine-linked glycosylation are preferably grown in a medium containing an osmotic

stabilizer. A preferred osmotic stabilizer is sorbitol supplemented into the medium at a concentration between 0.1 M and 1.5 M, preferably at 0.5 M or 1.0 M. Cultured mammalian cells are generally cultured in commercially available serum-containing or serum-free media. Selection of a medium appropriate for the particular host cell used is within the level of ordinary skill in the art.

Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler *et al.*, *Cell* 14:725, 1978; Corsaro and Pearson, *Somatic Cell Genetics* 7:603, 1981; Graham and Van der Eb, *Virology* 52:456, 1973), electroporation (Neumann *et al.*, *EMBO J.* 1:841-45, 1982) and DEAE-dextran mediated 'transfection (Ausubel *et al.*, (eds), *Current Protocols in Molecular Biology*, John Wiley and Sons, Inc., NY, 1987), which are incorporated herein by reference. Cationic lipid transfection using commercially available reagents, including the Boehringer Mannheim TRANSFECTION-REAGENT (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethyl ammoniummethylsulfate; Boehringer Mannheim, Indianapolis, IN) or LIPOFECTIN reagent (N-[1-(2,3-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride and dioleoyl phosphatidylethanolamine; GIBCO-BRL, Gaithersburg, MD) using the manufacturer-supplied directions, may also be used. A preferred mammalian expression plasmid is Zem229R (deposited under the terms of the Budapest Treaty with American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD on September 28, 1993 as an *E. coli* HB101 transformant and assigned Accession Number 69447). The production of recombinant proteins in cultured mammalian cells is disclosed, for example, by Levinson *et al.*, U.S. Patent No. 4,713,339; Hagen *et al.*, U.S. Patent No. 4,784,950; Palmiter *et al.*, U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134, which are incorporated herein by reference. Preferred cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), DG44, and 293 (ATCC No. CRL 1573; Graham *et al.*, *J. Gen. Virol.* 36:59-72, 1977) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Rockville, Maryland. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include

those from metallothionein genes (U.S. Patents Nos. 4,579,821 and 4,601,978, which are incorporated herein by reference) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants." Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems may also be used to increase the expression level of the gene of interest, a process referred to as "amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used.

The soluble, fused MHC:peptide complexes of the present invention can be purified by first isolating the polypeptides from the cells followed by conventional purification methods, such as by ion-exchange and partition chromatography as described by, for example, Coy *et al.* (Peptides Structure and Function, Pierce Chemical Company, Rockford, IL, pp 369-72, 1983) or by reverse-phase chromatography as described, for example, by Andreu and Merrifield (*Eur. J. Biochem.* 164: 585-90, 1987), or by HPLC as described, for example, by Kofod *et al.* (*Int. J. Peptide and Protein Res.* 32.: 436-40, 1988). Additional purification can be achieved by additional conventional purification means, such as liquid chromatography, gradient centrifugation, and gel electrophoresis, among others. Methods of protein purification are known in the art (see generally, Scopes, R., *Protein Purification*, Springer-Verlag, NY, 1982, which is incorporated by reference herein) and can be applied to the purification of the recombinant polypeptides described herein. Soluble, fused MHC heterodimer:peptide complexes of at least about 50% purity are preferred, at least about 70-80% purity more preferred, and about 95-99% or more purity most preferred, particularly for pharmaceutical uses. Once purified, either

partially or to homogeneity, as desired, the soluble, fused MHC heterodimer:peptide complexes may then be used diagnostically or therapeutically, as further described below.

#### **Methods of using the MHC class II heterodimer:peptide complexes**

5           The soluble, fused MHC heterodimer:peptide complexes of the present invention may be used within methods for down-regulating parts of the immune system that are reactive in autoimmune diseases. The soluble, fused MHC heterodimer:peptide complexes of the present invention are contemplated to be advantageous for use as immunotherapeutics to induce immunological tolerance or nonresponsiveness (anergy) in  
10 patients predisposed to mount or already mounting an immune response those particular autoantigens. A patient having or predisposed to a particular autoimmune disease is identified and MHC type is determined by methods known in the art. The patient's T cells can be examined *in vitro* to determine autoantigenic peptide(s) recognized by the patient's autoreactive T cells using complexes and methods described herein. The patient  
15 can then be treated with complexes of the invention. Such methods will generally include administering soluble, fused MHC heterodimer:peptide complex in an amount sufficient to lengthen the time period before onset of the autoimmune disease and/or to ameliorate or prevent that disease. Soluble, fused MHC heterodimer:peptide complexes of the present invention are therefore contemplated to be advantageous for use in both  
20 therapeutic and diagnostic applications related to auto immune diseases.

Kits can also be supplied for therapeutic or diagnostic uses. Thus, the subject composition of the present invention may be provided, usually in a lyophilized form, in a container. The soluble, fused MHC heterodimer:peptide complex is included in the kits with instructions for use, and optionally with buffers, stabilizers, biocides, and  
25 inert proteins. Generally, these optional materials will be present at less than about 5% by weight, based on the amount of soluble, fused MHC heterodimer:peptide complex, and will usually be present in a total amount of at least about 0.001% by weight, based on the soluble, fused MHC heterodimer:peptide complex concentration. It may be desirable to include an inert extender or excipient to dilute the active ingredients, where the excipient  
30 may be present in from about 1 to 99% weight of the total composition.

Within one aspect of the present invention, soluble, fused MHC heterodimer:peptide complexes are utilized to prepare antibodies for diagnostic or

therapeutic uses. As used herein, the term "antibodies" includes polyclonal antibodies, monoclonal antibodies, antigen-binding fragments thereof such as  $F(ab')_2$  and Fab fragments, as well as recombinantly produced binding partners. These binding partners incorporate the variable or CDR regions from a gene which encodes a specifically binding antibody. The affinity of a monoclonal antibody or binding partner may be readily determined by one of ordinary skill in the art (see, Scatchard, *Ann. NY Acad. Sci.* 51: 660-72, 1949)

Methods for preparing polyclonal and monoclonal antibodies have been well described in the literature (see, for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual, Second Edition*, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., *Monoclonal Hybridoma Antibodies: Techniques and Applications*, CRC Press, Ind., Boca Raton, FL, 1982, which is incorporated herein by reference). As would be evident to one of ordinary skill in the art, polyclonal antibodies may be generated from a variety of warm-blooded animals, such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, or rats, for example. The immunogenicity of the soluble, fused MHC heterodimer:peptide complexes may be increased through the use of an adjuvant, such as Freund's complete or incomplete adjuvant. A variety of assays known to those skilled in the art may be utilized to detect antibodies which specifically bind to a soluble, fused MHC heterodimer:peptide complex. Exemplary assays are described in detail in *Antibodies: A Laboratory Manual*, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radio-immunoassays, radio-immunoprecipitations, enzyme-linked immuno-sorbent assays, dot blot assays, inhibition or competition assays, and sandwich assays.

Additional techniques for the preparation of monoclonal antibodies may be utilized to construct and express recombinant monoclonal antibodies. Briefly, mRNA is isolated from a B cell population and used to create heavy and light chain immunoglobulin cDNA expression libraries in a suitable vector such as the  $\lambda$ IMMUNOZAP(H) and  $\lambda$ IMMUNOZAP(L) vectors, which may be obtained from Stratagene Cloning Systems (La Jolla, CA). These vectors are then screened individually or are co-expressed to form Fab fragments or antibodies (Huse *et al.*, *Science* 246 1275-81, 1989; Sastry *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 5728-32, 1989). Positive plaques

are subsequently converted to a non-lytic plasmid which allows high level expression of monoclonal antibody fragments in *E. coli*.

Antibodies of the present invention may be produced by immunizing an animal selected from a wide variety of warm-blooded animals, such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats, with a recombinant soluble, fused MHC heterodimer:peptide complex. Serum from such animals are a source of polyclonal antibodies. Alternatively antibody producing cells obtained from the immunized animals are immortalized and screened. As the generation of human monoclonal antibodies to a human antigen, such as a soluble, fused MHC heterodimer:peptide complex, may be difficult with conventional immortalization techniques, it may be desirable to first make non-human antibodies. Using recombinant DNA techniques, the antigen binding regions of the non-human antibody is transferred to the corresponding site of a human antibody coding region to produce a substantially human antibody molecules. Such methods are generally known in the art and are described in, for example, U.S. Patent No. 4,816,397, and EP publications 173,494 and 239,400, which are incorporated herein by reference.

In another aspect of the invention, the soluble, fused MHC heterodimer:peptide complexes can be used to clone T cells which have specific receptors for the soluble, fused MHC heterodimer:peptide complex. Once the soluble, fused MHC heterodimer:peptide complex-specific T cells are isolated and cloned using techniques generally available to the skilled artisan, the T cells or membrane preparations thereof can be used to immunize animals to produce antibodies to the soluble, fused MHC heterodimer:peptide complex receptors on T cells. The antibodies can be polyclonal or monoclonal. If polyclonal, the antibodies can be murine, lagomorph, equine, ovine, or from a variety of other mammals. Monoclonal antibodies will typically be murine in origin, produced according to known techniques, or human, as described above, or combinations thereof, as in chimeric or humanized antibodies. The anti-soluble, fused MHC heterodimer:peptide complex receptor antibodies thus obtained can then be administered to patients to reduce or eliminate T cell subpopulations that display such receptor. This T-cell population recognizes and participates in the immunological destruction of cells bearing the autoantigenic peptide in an individual predisposed to or already suffering from a disease, such as an autoimmune disease related to the autoantigenic peptide.

The coupling of antibodies to solid supports and their use in purification of proteins is well known in the literature (*see, for example, Methods in Molecular Biology. Vol. 1*, Walker (Ed.), Humana Press, New Jersey, 1984, which is incorporated by reference herein in its entirety). Antibodies of the present invention may be used as a  
5 marker reagent to detect the presence of MHC heterodimer:peptide complexes on cells or in solution. Such antibodies are also useful for Western analysis or immunoblotting, particularly of purified cell-secreted material. Polyclonal, affinity purified polyclonal, monoclonal and single chain antibodies are suitable for use in this regard. In addition, proteolytic and recombinant fragments and epitope binding domains can be used herein.  
10 Chimeric, humanized, veneered, CDR-replaced, reshaped or other recombinant whole or partial antibodies are also suitable.

### Pharmaceutical compositions

Pharmaceutically acceptable carriers are determined in part by the  
15 particular composition being administered (*e.g.*, nucleic acid, protein) as by the particular method used to administer the composition. Accordingly, there are a wide variety of suitable formulations of pharmaceutical compositions of the present invention (*see, e.g., Remington's Pharmaceutical Sciences* (17<sup>th</sup> ed., 1989). Administration can be in any convenient manner, *e.g.*, by injection, oral administration, inhalation, or transdermal  
20 application.

Formulations suitable for oral administration can consist of (a) liquid solutions, such as an effective amount of the packaged nucleic acid or polypeptide suspended in diluents, such as water, saline or PEG 400; (b) capsules, sachets or tablets, each containing a predetermined amount of the active ingredient, as liquids, solids,  
25 granules or gelatin; (c) suspensions in an appropriate liquid; and (d) suitable emulsions. Tablet forms can include one or more of lactose, sucrose, mannitol, sorbitol, calcium phosphates, corn starch, potato starch, microcrystalline cellulose, gelatin, colloidal silicon dioxide, talc, magnesium stearate, stearic acid, and other excipients, colorants, fillers, binders, diluents, buffering agents, moistening agents, preservatives, flavoring agents,  
30 dyes, disintegrating agents, and pharmaceutically compatible carriers. Lozenge forms can comprise the active ingredient in a flavor, *e.g.*, sucrose, as well as pastilles comprising the active ingredient in an inert base, such as gelatin and glycerin or sucrose and acacia

emulsions, gels, and the like containing, in addition to the active ingredient, carriers known in the art.

The compound of choice, alone or in combination with other suitable components, can be made into aerosol formulations (*i.e.*, they can be “nebulized”) to be administered via inhalation. Aerosol formulations can be placed into pressurized acceptable propellants, such as dichlorodifluoromethane, propane, nitrogen, and the like.

Formulations suitable for parenteral administration, such as, for example, by intraarticular (in the joints), intravenous, intramuscular, intradermal, intraperitoneal, and subcutaneous routes, include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives. In the practice of this invention, compositions can be administered, for example, by intravenous infusion, orally, topically, intraperitoneally, intravesically or intrathecally. Parenteral administration and intravenous administration are the preferred methods of administration. The formulations of compounds can be presented in unit-dose or multi-dose sealed containers, such as ampules and vials.

Injection solutions and suspensions can be prepared from sterile powders, granules, and tablets of the kind previously described. Cells transduced by nucleic acids for *ex vivo* therapy can also be administered intravenously or parenterally as described above.

The polypeptides of the invention are administered prophylactically or to an individual already suffering from the disease. The compositions are administered to a patient in an amount sufficient to elicit an effective immune response. An amount adequate to accomplish this is defined as “therapeutically effective dose” or “immunogenically effective dose.” Amounts effective for this use will depend on, *e.g.*, the peptide composition, the manner of administration, the stage and severity of the disease being treated, the weight and general state of health of the patient, and the judgment of the prescribing physician, but generally range for the initial immunization dose (that is for therapeutic or prophylactic administration) from about 0.01 mg to about 50 mg per 70 kilogram patient, more commonly from about 0.5-1 mg to about 10-15 mg per 70 kg of body weight. Boosting dosages are typically from about 0.01 mg to about 50



mg of peptide, more commonly about 0.5-1 mg to about 10-15 mg, using a boosting regimen over weeks to months depending upon the patient's response and condition. A suitable protocol would include injection at time 0, 2, 6, 8, 10 and 14 weeks, followed by booster injections at 24 and 28 weeks. Booster injections can be from one, two, three, four, five or more. Initial and booster injection amounts and timing are determined based on the judgment of the physician and the antigen being administered. In one embodiment, the initial and booster dose is 1.3 mg, 4 mg, or 13 mg, administered via intramuscular injection, with at least one and up to 3 booster injections at 8 week intervals, or at least one and up to 4 booster injections at 6 week intervals.

The therapeutic methods of the present invention may involve oral tolerance (Weiner *et al.*, *Nature* 376: 177-80, 1995), or intravenous tolerance, for example. Tolerance can be induced in mammals, although conditions for inducing such tolerance will vary according to a variety of factors. To induce immunological tolerance in an adult susceptible to or already suffering from an autoantigen-related disease such as IDDM, the precise amounts and frequency of administration will also vary. For instance for adults about 20-80  $\mu\text{g/kg}$  can be administered by a variety of routes, such as parenterally, orally, by aerosols, intradermal injection, and the like. For neonates, tolerance can be induced by parenteral injection or more conveniently by oral administration in an appropriate formulation. The precise amount administered, and the mode and frequency of dosages, will vary.

The soluble, fused MHC heterodimer:peptide complexes will typically be more tolerogenic when administered in a soluble form, rather than in an aggregated or particulate form. Persistence of a soluble, fused MHC heterodimer:peptide complex of the invention is generally needed to maintain tolerance in an adult, and thus may require more frequent administration of the complex, or its administration in a form which extends the half-life of the complex. See for example, Sun *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 10795-99, 1994.

Within another aspect of the invention, a pharmaceutical composition is provided which comprises a soluble, fused MHC heterodimer:peptide complex of the present invention contained in a pharmaceutically acceptable carrier or vehicle for parenteral, topical, oral, or local administration, such as by aerosol or transdermally, for prophylactic and/or therapeutic treatment, according to conventional methods. The

composition may typically be in a form suited for systemic injection or infusion and may, as such, be formulated with sterile water or an isotonic saline or glucose solution.

Formulations may further include one or more diluents, fillers, emulsifiers, preservatives, buffers, excipients, and the like, and may be provided in such forms as liquids, powders,  
5 emulsions, suppositories, liposomes, transdermal patches and tablets, for example.

Pharmaceutical compositions of the present invention are administered at daily to weekly intervals. An "effective amount" of such a pharmaceutical composition is an amount that provides a clinically significant decrease in a deleterious T cell-mediated immune response to an autoantigen, for example, those associated with IDDM, or  
10 provides other pharmacologically beneficial effects. Such amounts will depend, in part, on the particular condition to be treated, age, weight, and general health of the patient, and other factors evident to those skilled in the art. Preferably the amount of the soluble, fused MHC heterodimer:peptide complex administered will be within the range of 20-80  $\mu\text{g/kg}$ . Compounds having significantly enhanced half-lives may be administered at  
15 lower doses or less frequently.

### Adjuvants

An immunostimulant refers to essentially any substance that enhances or potentiates an immune response (antibody and/or cell-mediated) to an exogenous antigen.

20 One preferred type of immunostimulant comprises an adjuvant. Many adjuvants contain a substance designed to protect the antigen from rapid catabolism, such as aluminum hydroxide or mineral oil, and a stimulator of immune responses, such as lipid A, *Bordetella pertussis* or *Mycobacterium tuberculosis* derived proteins. Certain adjuvants are commercially available as, for example, Freund's Incomplete Adjuvant and Complete  
25 Adjuvant (Difco Laboratories, Detroit, MI); Merck Adjuvant 65 (Merck and Company, Inc., Rahway, NJ); AS-2 (SmithKline Beecham, Philadelphia, PA); aluminum salts such as aluminum hydroxide gel (alum) or aluminum phosphate; salts of calcium, iron or zinc; an insoluble suspension of acylated tyrosine; acylated sugars; cationically or anionically derivatized polysaccharides; polyphosphazenes; biodegradable microspheres;  
30 monophosphoryl lipid A and quil A. Cytokines, such as GM-CSF, interleukin-2, -7, -12, and other like growth factors, may also be used as adjuvants.

The compositions may also include a *Mycobacterium* species CWS adjuvant, as described above. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the immunogenic peptide.

Certain adjuvants for eliciting a predominantly Th1-type response include, for example, a combination of monophosphoryl lipid A, preferably 3-de-O-acylated monophosphoryl lipid A, together with an aluminum salt. MPL<sup>®</sup> adjuvants are available from Corixa Corporation (Seattle, WA; *see*, for example, US Patent Nos. 4,436,727; 4,877,611; 4,866,034 and 4,912,094). CpG-containing oligonucleotides (in which the CpG dinucleotide is unmethylated) also induce a predominantly Th1 response. Such oligonucleotides are well known and are described, for example, in WO 96/02555, WO 99/33488 and U.S. Patent Nos. 6,008,200 and 5,856,462. Immunostimulatory DNA sequences are also described, for example, by Sato *et al.*, *Science* 273:352, 1996.

Another preferred adjuvant comprises a saponin, such as Quil A, or derivatives thereof, including QS21 and QS7 (Aquila Biopharmaceuticals Inc., Framingham, MA); Escin; Digitonin; or *Gypsophila* or *Chenopodium quinoa* saponins. Other preferred formulations include more than one saponin in the adjuvant combinations of the present invention, for example combinations of at least two of the following group comprising QS21, QS7, Quil A,  $\beta$ -escin, or digitonin.

Alternatively the saponin formulations may be combined with vaccine vehicles composed of chitosan or other polycationic polymers, polylactide and polylactide-co-glycolide particles, poly-N-acetyl glucosamine-based polymer matrix, particles composed of polysaccharides or chemically modified polysaccharides, liposomes and lipid-based particles, particles composed of glycerol monoesters, etc. The saponins may also be formulated in the presence of cholesterol to form particulate structures such as liposomes or ISCOMs. Furthermore, the saponins may be formulated together with a polyoxyethylene ether or ester, in either a non-particulate solution or suspension, or in a particulate structure such as a paucilamellar liposome or ISCOM. The saponins may also be formulated with excipients such as Carbopol<sup>R</sup> to increase viscosity, or may be formulated in a dry powder form with a powder excipient such as lactose.

In one embodiment, the adjuvant system includes the combination of a monophosphoryl lipid A and a saponin derivative, such as the combination of QS21 and 3D-MPL<sup>®</sup> adjuvant, as described in WO 94/00153, or a less reactogenic composition

where the QS21 is quenched with cholesterol, as described in WO 96/33739. Other formulations comprise an oil-in-water emulsion and tocopherol. Another adjuvant formulation employs QS21, 3D-MPL<sup>®</sup> adjuvant and tocopherol in an oil-in-water emulsion is described in WO 95/17210.

5 Another enhanced adjuvant system involves the combination of a CpG-containing oligonucleotide and a saponin derivative particularly the combination of CpG and QS21 as disclosed in WO 00/09159. Preferably the formulation additionally comprises an oil in water emulsion and tocopherol.

Additional illustrative adjuvants for use in the pharmaceutical  
10 compositions of the invention include Montanide ISA 720 (Seppic, France), SAF (Chiron, California, United States), ISCOMS (CSL), MF-59 (Chiron), the SBAS series of adjuvants (*e.g.*, SBAS-2 or SBAS-4, available from SmithKline Beecham, Rixensart, Belgium), Detox (Enhanzyn<sup>®</sup>) (Corixa, Hamilton, MT), RC-529 (Corixa, Hamilton, MT) and other aminoalkyl glucosaminide 4-phosphates (AGPs), such as those described in  
15 pending U.S. Patent Application Serial Nos. 08/853,826 and 09/074,720, the disclosures of which are incorporated herein by reference in their entireties, and polyoxyethylene ether adjuvants such as those described in WO 99/52549A1.

Other preferred adjuvants include adjuvant molecules of the general formula (I):  $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_n\text{-A-R}$ ,  
20 wherein,  $n$  is 1-50,  $A$  is a bond or  $-\text{C}(\text{O})-$ ,  $R$  is  $\text{C}_{1-50}$  alkyl or Phenyl  $\text{C}_{1-50}$  alkyl.

One embodiment of the present invention consists of a vaccine formulation comprising a polyoxyethylene ether of general formula (I), wherein  $n$  is between 1 and 50, preferably 4-24, most preferably 9; the  $R$  component is  $\text{C}_{1-50}$ , preferably  $\text{C}_4\text{-C}_{20}$  alkyl and most preferably  $\text{C}_{12}$  alkyl, and  $A$  is a bond. The concentration of the polyoxyethylene  
25 ethers should be in the range 0.1-20%, preferably from 0.1-10%, and most preferably in the range 0.1-1%. Preferred polyoxyethylene ethers are selected from the following group: polyoxyethylene-9-lauryl ether, polyoxyethylene-9-stearyl ether, polyoxyethylene-8-stearyl ether, polyoxyethylene-4-lauryl ether, polyoxyethylene-35-lauryl ether, and polyoxyethylene-23-lauryl ether. Polyoxyethylene ethers such as polyoxyethylene lauryl  
30 ether are described in the Merck index (12<sup>th</sup> edition: entry 7717). These adjuvant molecules are described in WO 99/52549.

The polyoxyethylene ether according to the general formula (I) above may, if desired, be combined with another adjuvant. For example, a preferred adjuvant combination is preferably with CpG as described in the pending UK patent application GB 9820956.2.

5

#### Liposome-, Nanocapsule-, and Microparticle-Mediated Delivery

In certain embodiments, the inventors contemplate the use of liposomes, nanocapsules, microparticles, microspheres, lipid particles, vesicles, and the like, for the introduction of the compositions of the present invention into the subjects. In particular,  
10 the compositions of the present invention may be formulated for delivery either encapsulated in a lipid particle, a liposome, a vesicle, a nanosphere, or a nanoparticle or the like.

Such formulations may be preferred for the introduction of pharmaceutically-acceptable formulations of the compositions disclosed herein. The  
15 formation and use of liposomes is generally known to those of skill in the art (*see*, for example, Couvreur *et al.*, 1977; Couvreur, 1988; Lasic, 1998; which describes the use of liposomes and nanocapsules in the targeted antibiotic therapy for intracellular bacterial infections and diseases). Recently, liposomes were developed with improved serum stability and circulation half-times (Gabizon & Papahadjopoulos, 1988; Allen and Choun,  
20 1987; U. S. Patent 5,741,516, specifically incorporated herein by reference in its entirety). Further, various methods of liposome and liposome-like preparations as potential drug carriers have been reviewed (Takakura, 1998; Chandran *et al.*, 1997; Margalit, 1995; U. S. Patent 5,567,434; U. S. Patent 5,552,157; U. S. Patent 5,565,213; U. S. Patent 5,738,868 and U. S. Patent 5,795,587, each specifically incorporated herein by reference  
25 in its entirety).

Liposomes have been used effectively to introduce genes, drugs (Heath & Martin, 1986; Heath *et al.*, 1986; Balazsovits *et al.*, 1989; Fresta & Puglisi, 1996), radiotherapeutic agents (Pikul *et al.*, 1987), enzymes (Imaizumi *et al.*, 1990a; Imaizumi *et al.*, 1990b), viruses (Faller & Baltimore, 1984), transcription factors and allosteric  
30 effectors (Nicolau & Gersonde, 1979) into a variety of cultured cell lines and animals. In addition, several successful clinical trials examining the effectiveness of liposome-mediated drug delivery have been completed (Lopez-Berestein *et al.*, 1985a; 1985b;

Coune, 1988; Sculier *et al.*, 1988). Furthermore, several studies suggest that the use of liposomes is not associated with autoimmune responses, toxicity or gonadal localization after systemic delivery (Mori & Fukatsu, 1992).

5 Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs). MLVs generally have diameters of from 25 nm to 4  $\mu$ m. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 Å, containing an aqueous solution in the core.

10 Liposomes bear resemblance to cellular membranes and are contemplated for use in connection with the present invention as carriers for the compositions. They are widely suitable as both water- and lipid-soluble substances can be entrapped, *i.e.*, in the aqueous spaces and within the bilayer itself, respectively. It is possible that the drug-bearing liposomes may even be employed for site-specific delivery of active agents by selectively modifying the liposomal formulation.

15 In addition to the teachings of Couvreur *et al.* (1977, 1988), the following information may be utilized in generating liposomal formulations. Phospholipids can form a variety of structures other than liposomes when dispersed in water, depending on the molar ratio of lipid to water. At low ratios the liposome is the preferred structure. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations. Liposomes can show low permeability to ionic and polar substances, but at elevated temperatures undergo a phase transition which markedly alters their permeability. The phase transition involves a change from a closely packed, ordered structure, known as the gel state, to a loosely packed, less-ordered structure, known as the fluid state. This occurs at a characteristic phase-transition temperature and results in an increase in permeability to ions, sugars and drugs.

25 In addition to temperature, exposure to proteins can alter the permeability of liposomes. Certain soluble proteins, such as cytochrome c, bind, deform and penetrate the bilayer, thereby causing changes in permeability. Cholesterol inhibits this penetration of proteins, apparently by packing the phospholipids more tightly. It is contemplated that the most useful liposome formations for antibiotic and inhibitor delivery will contain cholesterol.

The ability to trap solutes varies between different types of liposomes. For example, MLVs are moderately efficient at trapping solutes, but SUVs are extremely inefficient. SUVs offer the advantage of homogeneity and reproducibility in size distribution. However, a compromise between size and trapping efficiency is offered by large unilamellar vesicles (LUVs). These are prepared by ether evaporation and are three to four times more efficient at solute entrapment than MLVs.

In addition to liposome characteristics, an important determinant in entrapping compounds is the physicochemical properties of the compound itself. Polar compounds are trapped in the aqueous spaces and nonpolar compounds bind to the lipid bilayer of the vesicle. Polar compounds are released through permeation or when the bilayer is broken, but nonpolar compounds remain affiliated with the bilayer unless it is disrupted by temperature or exposure to lipoproteins. Both types show maximum efflux rates at the phase transition temperature.

Liposomes interact with cells *via* four different mechanisms: endocytosis by phagocytic cells of the reticuloendothelial system such as macrophages and neutrophils; adsorption to the cell surface, either by nonspecific weak hydrophobic or electrostatic forces, or by specific interactions with cell-surface components; fusion with the plasma cell membrane by insertion of the lipid bilayer of the liposome into the plasma membrane, with simultaneous release of liposomal contents into the cytoplasm; and by transfer of liposomal lipids to cellular or subcellular membranes, or vice versa, without any association of the liposome contents. It often is difficult to determine which mechanism is operative and more than one may operate at the same time.

The fate and disposition of intravenously injected liposomes depend on their physical properties, such as size, fluidity, and surface charge. They may persist in tissues for hours or days, depending on their composition, and half lives in the blood range from minutes to several hours. Larger liposomes, such as MLVs and LUVs, are taken up rapidly by phagocytic cells of the reticuloendothelial system, but physiology of the circulatory system restrains the exit of such large species at most sites. They can exit only in places where large openings or pores exist in the capillary endothelium, such as the sinusoids of the liver or spleen. Thus, these organs are the predominant site of uptake. On the other hand, SUVs show a broader tissue distribution but still are sequestered highly in the liver and spleen. In general, this *in vivo* behavior limits the potential

targeting of liposomes to only those organs and tissues accessible to their large size. These include the blood, liver, spleen, bone marrow, and lymphoid organs.

Targeting is generally not a limitation in terms of the present invention. However, should specific targeting be desired, methods are available for this to be accomplished. Antibodies may be used to bind to the liposome surface and to direct the antibody and its drug contents to specific receptors located on a particular cell-type surface. Carbohydrate determinants (glycoprotein or glycolipid cell-surface components that play a role in cell-cell recognition, interaction and adhesion) may also be used as recognition sites as they have potential in directing liposomes to particular cell types. Mostly, it is contemplated that intravenous injection of liposomal preparations would be used, but other routes of administration are also conceivable.

Alternatively, the invention provides for pharmaceutically-acceptable nanocapsule formulations of the compositions of the present invention. Nanocapsules can generally entrap compounds in a stable and reproducible way (Henry-Michelland *et al.*, 1987; Quintanar-Guerrero *et al.*, 1998; Douglas *et al.*, 1987). To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1  $\mu\text{m}$ ) should be designed using polymers able to be degraded *in vivo*. Biodegradable polyalkylcyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention. Such particles may be easily made, as described (Couvreux *et al.*, 1980; 1988; zur Muhlen *et al.*, 1998; Zambaux *et al.* 1998; Pinto-Alphandry *et al.*, 1995 and U. S. Patent 5,145,684, specifically incorporated herein by reference in its entirety).

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.



The following example is provided by way of illustration only and not by way of limitation. Those of skill in the art will readily recognize a variety of noncritical parameters that could be changed or modified to yield essentially similar results.

5

## EXAMPLES

Example 1: Construction of DNA sequences encoding human soluble, fused MHC heterodimer:peptide complexes, HLA-DR4 molecules(CO563 and CO564).

DNA constructs encoding gp39  $\beta 1/\alpha 1$  human molecules were prepared from a cDNA encoding the gp39 peptide fused to the  $\beta 1/\alpha 1$  domains of HLA-DR4 according to standard techniques. For the production of "empty"  $\beta 1/\alpha 1$  DR4 molecules, a cDNA encoding the fused  $\beta 1/\alpha 1$  domains of HLA-DR4 was prepared using cloned  $\alpha$  and  $\beta$  chains from DR4.

Amino acid sequence of the gp39- $\beta 1/\alpha 1$  HLA-DR4 human single chain molecule (linkers are shown in bold)

15 MGD~~T~~GRSFTLASSETGVGASGGGGSGGGGDTRPRFLEQVKHECH  
FFNGTERVRFLDRYFYHQEEYVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQ  
KRAAVDTYCRHNYGVGESFTVQRRGGIKEEHVIIQAEFYLNPDQSGEFMFDFDG  
DEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPIT  
N\*

20 aa1 – aa4: leader sequence  
aa5' - aa18: gp39 peptide  
aa19 - aa28: linker  
aa29 - aa122: HLA-DR4  $\beta 1$  domain  
aa123 – aa124: linker  
25 aa125 – aa 208: HLA-DR4  $\alpha 1$  domain

Amino acid sequence of the "empty"  $\beta 1/\alpha 1$  HLA-DR4 human single chain molecule (linkers in bold):

MGDTRPRFLEQVKHECHFFNGTERVRFLDRYFYHQEEYVRFDSDVGEYRAVTEL  
GRPDAEYWNSQRDLLEQKRAAVDTYCRHNYGVGESFTVQRRGGIKEEHVIIQAE  
30 FYLNPDQSGEFMFDFDGDEIFHVDMAKKETVWRLEEFGRFASFEAQGALANIAV  
DKANLEIMTKRSNYTPITN\*

aa 1 – aa95: HLA-DR4  $\beta 1$  domain

aa96- aa97: linker

aa98- aa181: HLA-DR4  $\alpha 1$  domain

For the production of recombinant proteins, the bacteria (pLysS) were grown in LB (containing ampicillin (50  $\mu\text{g} / \text{ml}$ ) and chloramphenicol (5  $\mu\text{g} / \text{ml}$ )) at 37°C until OD 600 = 0.5. IPTG was added at the final concentration of 0.5 mM final and the bacteria were further incubated for 3 hours at 37°C with shaking. The bacteria were centrifuged at 4°C, 4000xg for 20 min and the pellet was frozen at -80°C. The following day, the pellet was resuspended in 40 ml of lysis buffer (50 mM Tris-HCl pH 8, 50 mM NaCl, 2 mM EDTA, 1 protease inhibitor cocktail tablet, 1% Triton X100 and 1% deoxycholate), and incubated for 1 hour at 4°C under continuous agitation with a magnetic stirrer. The sample was then homogenized using a French Press with a 16,000 psi setting, and centrifuged at 4°C, 9000g for 20 min. The pellet was then resuspended in 30 ml of lysis buffer without Triton and deoxycholate and centrifuged at 4°C, 9000 g for 20 min. The new pellet was resuspended in 10 ml of 20 mM ethanolamine/6 M urea pH 10, and eventually frozen at -80°C. The recombinant protein was then purified by FPLC ion-exchange chromatography using Source 30Q anion-exchange media in an XK26/20 column using a step gradient going from 1 mM to 1 M NaCl in 20 mM ethanolamine/6M urea pH 10. Fractions were analyzed by SDS/PAGE and those corresponding to the proteins of interest are pooled and dialyzed against PBS 1X.

#### Example 2: Production of additional single chain constructs

Additional constructs with different composition and length of the 2<sup>nd</sup> linker (between  $\beta 1$  and  $\alpha 1$ ) were engineered by using standard techniques using CO567 as the template. Specifically, PCR primers were designed to replace the old sequence in CO567 with the new sequence. For example, to make CO581, the primers were designed with the following sequences (note these primers were phosphorylated at 5').

Primer 1:

5' pCACCAGGAGGAGAGCCGCCACGCCGGTCTCGCTGG

Primer 2:

5' pGACCACCTGGATCTGGGGACACCCGACCACGTTTC

PCR reaction (100  $\mu\text{l}$ ) was made of the following components: 2  $\mu\text{l}$  CO567 (80 ng) as template, 2  $\mu\text{l}$  each of primer 1 and primer 2 (10  $\mu\text{M}$ ), 2  $\mu\text{l}$  of dNTP

mix (20 mM each), 10 µl of 10X pfu buffer, and 80 µl of sterile water. After all the components were mixed, 2 µl of Turbo pfu (5U total ) was added, mixed and put on PCR machine. The PCR cycles has a pre-denaturation at 95°C for 30 sec, then 10 cycles of 95°C for 30°C, 60°C for 1 min, and 72°C for 7 min. Then another 22 cycles of 95°C for 30°C, 65°C for 1 min and 72°C for 7 min, followed by a final 10 min at 72°C.

The PCR mixture was digested with 2 µl of DpnI (10U) for 2h at 37°C. Then the PCR product at ~6 kb was purified from agarose gel after electrophoresis. The purified PCR product was ligated by T4 DNA ligase for 1 h at room temperature then used to transform into NovaBlue (Novagen)competent cells by standard protocol. Cells were plated on LB (+Carb) and grow overnight at 37°C.

Next day, about a dozen single colony from the transformation were randomly picked for overnight culture in 5 ml LB (+Carb) at 37°C. Plasmids from these culture were purified with Wizard Miniprep kit, and analyzed by Xho I digestion. A few plasmids that passed the Xho I digestion were further confirmed by DNA sequencing.

To express the recombinant proteins, a clone with confirmed DNA sequence was used to transform BL21(DE3)CodonPlus-RIL (Stratagene) by standard transformation protocol and plated on LB (Carb+Cam) plates overnight at 37°C. Next morning, a single colony was picked to inoculate 100 ml LB (+Carb +Cam) and the culture was grown till OD reach between 0.8-1.0, and stored overnight at 4°C. Next day, the culture was pellet down and used to inoculate into 2xYT (+Carb+Cam) at ratio of 25 ml culture per liter new media. These large cultures were grown at 37°C till OD=0.5-0.6, and IPTG was added to induce recombinant protein at 37°C for 3 h. The induced cultures were pellet and stored at -80°C till purification.

Table 1 provides a listing of various constructs made according to the invention.

52  
TABLE I

Construct	peptide	upstream linker	downstream linker
C0523	yes	GGGG	GG
C0543	none	none	GG
C0563	yes	ASGGGSGGG	GG
C0567	yes	ASGGGSGGG	TSGGGGSGGGGSSS
C0580	yes	ASGGGSGGG	GSPGGGGSGGGPGS
C0581	yes	ASGGGSGGG	GSPPGGPPGS
C0582	yes	ASGGGSGGG	GSPGGGGPGS
C0583	yes	ASGGGSGGG	TSGGGGS
C0584	yes	ASGGGSGGG	SGGSGGS
C0585	yes	ASGGGSGGG	FDAPSPLP
C0586	none	none	TSGGGGSGGGGSSS
C0587	none	none	GSPGGGGSGGGPGS
C0588	none	none	GSPPGGPPGS
C0589	none	none	GSPGGGGPGS
C0590	none	none	TSGGGGS
C0591	none	none	SGGSGGS
C0592	none	none	FDAPSPLP
C0593	yes	ASGGGSGGG	VYPEVTV
C0594	none	none	VYPEVTV
C0595	yes	ASGGGSGGG	GGGG
C0596	yes	ASGGGSGGG	GGGGS
C0597	yes	ASGGGSGGG	GGGSGG

Example 3: Four classes of novel linkers for MHC class II single chain molecules

Inspection of circular dichroism spectra of purified, refolded single chain constructs indicated that novel linkers could be used for the constructs. The atomic structures of the various murine and human MHC class II molecules, as determined by X-ray crystallography, indicated that these molecules have a high degree of structural similarity. The circular dichroism results were consistent with two folded molecules of clearly different secondary structure. Careful inspection of the structures reveals that the human MHC has a longer distance between chains than the equivalent murine molecule. This results led to proposing longer linkers between the chains which would contain flexible residues (e.g. alanine or glycine) and polar residues (e.g. serine and threonine). These constructs make up the first class of linkers. To inhibit the continuation of secondary structure across the linker, prolines were added to bracket the linkers. These prolines are known to inhibit the formation of alpha helices and beta sheets. These linkers make up the second class of linkers disclosed here.

Next, flexible regions present in the human MHC and in the murine MHC could be used to make a linker by extending the region of interest and ligating the ends together. These are the third class of linkers. Finally, a combination of these types of linkers could also be used. These are the fourth class of linkers.

Example 4: Human MHC class II single chain molecule with murine linkers

Another linker has been suggested based on a combination of murine and human MHC class II single chain molecules. This fusion would incorporate linker residues from the functional murine single chain MHC class II molecule and the alpha and beta chains of the human molecule. The protein sequence of the single chain molecule is provided below (the linker residues from the mouse construct are in bold):

MGDTGRSFTLASSETGVGASGGGGSGGGGDTRPRFLEQVKHECHFFNGTERVRF  
LDRYFYHQEEYVRFDSDVGEYRAVTELGRPDAEYWNSQKDLLEQKRAAVDTYC  
RHNYGVGESFTV**LRRLGGEDDEAD**HHVIIQAEFYLNPDQSGEFMDFDGDGDEIFH  
VDMAKKETVWRLEEFGRFASFEAQGALANIAVDKANLEIMTKRSNYTPITN\*

Further MHC class II hybrid single chain molecules may be designed by fusing other portions of the alpha chain and the beta chain together using linkers as described elsewhere. A properly folded molecule may be obtained by putting appropriate

linkers between portions of the human MHC class II which are proximal to each other as determined by visual inspection of the atomic coordinates of residues of the native MHC available in the publicly accessible protein structure database. These structures would predict possible fusion proteins which covalently attach any part of the beta chain

5 between residues 82 to 123 or between residues 148 to 164 to portions of the alpha chain such as the N-terminal residues, residues 79 to 84, or 92 to 106. The numbering system of residues in this example corresponds to those found in the coordinates of the structure described in: DESSEN, *et al. Immunity* 7:473 (1997). Other, homologous residues could be used to create equivalent constructs for genotypic and allelic variants of these

10 molecules e.g. equivalent residues in DR2 or such. DNAs for such hybrids would be prepared and expressed in a recombinant expression system by someone skilled in the art and could be assayed for structure and function in appropriate assays.

Example 5: Use of CD4 binding site(s) of MHC class II molecules as linkers for the

15 production of bioactive recombinant MHC class II-peptide complexes

HLA class II molecules present antigenic peptides to the T cell receptor of the CD4+ T lymphocytes and interact with CD4 during the antigen recognition process. Native MHC class II-peptide complexes have been shown to bind to MHC class II restricted and antigen specific TCRs on a particular T cell and induce T cell

20 nonresponsiveness. It is proposed that the CD4 binding site is important in the docking of MHC class II-peptide complex with the TCR and induce nonresponsiveness. Since the binding of CD4 to MHC class II-peptide is important in antigen presentation and/or induction of T cell nonresponsiveness, it is proposed that recombinant MHC class II-peptide molecules (truncated or whole) containing CD4 binding site will be biologically

25 active. Furthermore, a polypeptide fragment from MHC class II which binds the CD4, when used as a linker in preparation of MHC class II-peptide truncated molecules, provides resulting recombinant molecules that will be biologically active.

The following describes the concept of different linkers. Peptide is attached to a linker 1 (L1) which is attached to N-terminus of  $\beta$ 1 domain that is linked to

30 L2. L2 is linked to L3, which in turn is linked to N-terminus of  $\alpha$ 1 chain of MHC class II. Here L2 represents the human CD4 binding sequences. It should be noted that L2 could also be directly linked to N-terminus of  $\alpha$ 1 domain by completely deleting L3.

Specific examples of L1 and L3 are given in a examples. The sequences of L2 are given below. These sequences are applicable to most of the DR-Peptide molecules.

RNGQEEKAGVVSTGLI, RNGQETKAGVVSTGLI, YNQQEEKAGGVSTGLI,  
FRNGQEEKAGVVSTGLI, FRNGQETKAGVVSTGLI, FYNQQEEKAGGVSTGLI,  
and LNGQEEKAGMVSTGLI.

Example 6: I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.C $\kappa$  construct and activity

The “full anergix” single chain molecule, mouse I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.C $\kappa$ , was generated by overlap PCR using standard methodology. The molecules was expressed in 293T cells and baculoviral cells according to standard methodology, and purified according to standard methodology using affinity chromatography using goat-anti mouse antibodies. The structure of I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.C $\kappa$  is shown in Figure 1, and a comparison of its structure to a native murine molecule is shown in Figure 2. Figure 3 shows silver staining and western analysis of the recombinant proteins.

The amino acid sequence of the I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.C $\kappa$  shown in Figure 1 is as follows:

METDTLLLWVLLLWVPGSTGDFKNIVTPRTPPPASGGGGSGGGGDSERHFVFQF  
KGECYFTNGTQRIKRSVDRYIYNREEYLRFDSDVGEYRAVTELGRPDPPEYYNKQY  
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ASGGGGSGGGGADAAPTVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDG  
SERQNGVLNSWTDQDSKSTYSMSSTLTTLTKDEYERHNSYTCEATHKTSTSPIVK  
SFNRNEC

The *in vitro* activity of recombinant I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.C $\kappa$  was tested using MBP90-101 specific IAs restricted mouse T cell clone HS1. This clone was prepared by the immunization of SJL mice with the MBP90-101 peptide, followed by cloning out CD4<sup>+</sup> T cells by limited dilution techniques. These cells were maintained by stimulation every 10 days with irradiated SJF splenocytes and PBP90-101 peptide. The T

cells are activated by a combination of soluble recombinant I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck and plate bound anti-CD28 antibody (see Figure 4). T cell activation was assayed by  $^3$ H-thymidine incorporation according to standard methodology. Figure 5 shows the results of this assay.

5 MS is a T cell dependent autoimmune disease caused by localized demyelination in the central nervous system. Experimental autoimmune encephalomyelitis is a accepted animal model of MS. The following results demonstrate that administration of I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck reduces the incidence and severity of EAE. EAE was induced according to standard methodology according to the myelin  
10 model. Ten  $\mu$ g of recombinant I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck was given intravenously in 100  $\mu$ l of PBS at days 1, 4, 7, and 11 after disease induction. 12 days after immunization, animals are observed daily for the onset of neurological dysfunction. Disease is graded by trained technicians according to standard methods (see Figure 6). Mice are followed for up to 60-70 days. The date shown in Figure 7 demonstrate that administration of the  
15 recombinant I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck significantly reduced the incidence of myelin-induced EAE in SJL mice. In one experiment, 11 out of 20 mice developed EAE in the untreated group (55%), whereas only 2 out of 21 animal showed sign of the disease in the treated group (16.6%). Similarly, in another experiment, 12 out of 16 mice developed EAE in the untreated group (75%), while only 2 out of 16 developed EAE in the treated  
20 group (12.5%). Figure 8 shows histological data.

Example 7: Functional human anergix molecules optimized for *E. coli* expression with *E. coli* codons

Two human single chain MHC class II heterodimer molecules (CO528-AC  
25 and CO608-AC) have been optimized for *E. coli* expression using “artificial codons,” e.g., preferred *E. coli* codons encoding the mammalian protein. CO528-AC and CO608-AC were made according to standard PCR overlap technology.

Example 7: Recombinant MHC class II IAs.MBP.Ig multimeric complexes

30 Recombinant MHC class II (IAs)-peptide-Ig fusion complexes were constructed by fusing the mIgG leader, MBP 90-101, or MBP1-14 (as a control) to IAs single chain (b1b2a1a2), and mIgG.Ck, mIgG.CH1.H, mIgG.CH1.H.CH2, or



mIgG.CH1.H.CH2.CH3 with flexible linkers, according to standard methodology. The recombinant IAs fusion proteins were expressed in both mammalian and insect cells and detected by western analysis and ELISA. The overexpressed and secreted recombinant IAs fusion proteins from both human 293 cell cultures or from insect culture medium were purified by affinity chromatography. The purified dimeric and tetrameric recombinant IAs proteins have *in vitro* biological activity as assayed using an antigen-specific mouse T cell clone. The *in vivo* activity of the recombinant IAs fusion proteins were studied with the experimental autoimmune encephalomyelitis (EAE) model using susceptible SJL mice. In these EAE studies, recombinant IAs fusion protein was delivered on days 1, 4, 7, and 11 by I.V. injections after induction of the disease with myelin. The animals were then examined for neurological dysfunction. The results indicate that treatment with the recombinant IAs fusion proteins prevents mortality and significantly reduces paralysis induced by myelin homogenate in CFA. In conclusion, these studies suggest that the recombinant MHC class II fusion protein has therapeutic benefit as antigen-specific drugs for the treatment of autoimmune diseases.

#### Example 8: Synthesis of mouse model equivalent of CO608

Four forms of the murine MHC Class II IAs  $\beta 1\alpha 1$  with MBP peptide linkers analogous to CO608 human were made. These constructs can be used, e.g., as murine clinical control for human 608. mCO608 (mouse CO608), MCO608-A (lacking first four amino acids from beta 1 domain), *B. megaterium*-mCO608-A (expressed in *Bacillus megaterium*), and mCO608-B (first four amino acids replaced).

To make mCO608, the upstream linker of mouse CO521 (GGGS) was replaced with the human CO608 linker (ASGGGGSGGG) and the downstream linker of mouse CO521 (GG) was replaced with the downstream linker of CO608 (TSGGGGS), using PCR according to standard methodology. mCO608A was made from mCO608 using PCR according to standard methodology.

All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to one of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

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   tactggaaca gccagaagga cctcctggag cagaagcggg ccgcggtgga cacctactgc 240
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   ggcgggagcg gcggtggtcc tgggagtatc aaagaagaac atgtgatcat ccaggccgag 360
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ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 480  
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5 <210> C0586  
<211> 582  
<212> DNA  
<213> Homo sapiens

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15 ggcagcggcg gtggtggttc ctcgagtatc aaagaagaac atgtgatcat ccaggccgag 360  
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ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 480  
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20 <210> C0602  
<211> 186  
<212> PRT  
<213> Homo sapiens

25 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
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30 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
35 40 45

35 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
65 70 75 80

40 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Leu Arg Arg Leu  
85 90 95

45 Gly Gly Glu Asp Asp Glu Ala Asp His His Val Ile Ile Gln Ala Glu  
100 105 110

Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp  
115 120 125

50 Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp  
130 135 140

Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala  
145 150 155 160

55 Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys  
165 170 175

Arg Ser Asn Tyr Thr Pro Ile Thr Asn

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
5 10 15

55 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60

5 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly  
 85 90 95

10 Gly Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe  
 100 105 110

Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly  
 115 120 125

15 Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg  
 130 135 140

20 Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu  
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Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg  
 165 170 175

25 Ser Asn Tyr Thr Pro Ile Thr Asn  
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30 <210> CO599  
 <211> 184  
 <212> PRT  
 <213> Homo sapiens

35 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
 5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
 20 25 30

40 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60

45 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80

50 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly  
 85 90 95

Gly Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr  
 100 105 110

55 Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp  
 115 120 125

Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu  
 130 135 140



Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala  
 145 150 155 160

5 Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser  
 165 170 175

Asn Tyr Thr Pro Ile Thr Asn  
 180

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<210> CO594  
 <211> 187  
 <212> PRT  
 15 <213> Homo sapiens

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
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20 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
 20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45

25 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60

30 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Val  
 85 90 95

35 Tyr Pro Glu Val Thr Val Ile Lys Glu Glu His Val Ile Ile Gln Ala  
 100 105 110

Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe  
 115 120 125

40 Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val  
 130 135 140

Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly  
 145 150 155 160

Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr  
 165 170 175

50 Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
 180 185

55 <210> CO592  
 <211> 188  
 <212> PRT  
 <213> Homo sapiens

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys

5 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
35 40 45

10 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
65 70 75 80

15 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Phe  
85 90 95

Asp Ala Pro Ser Pro Leu Pro Ile Lys Glu Glu His Val Ile Ile Gln  
100 105 110

20 Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp  
115 120 125

25 Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr  
130 135 140

Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln  
145 150 155 160

30 Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met  
165 170 175

Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
180 185

35

<210> CO591  
<211> 187  
<212> PRT  
40 <213> Homo sapiens

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
5 10 15

45 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
20 25 30

Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
35 40 45

50 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
65 70 75 80

55 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Ser  
85 90 95

Gly Gly Ser Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala  
 100 105 110

5 Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe  
 115 120 125

Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val  
 130 135 140

10 Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly  
 145 150 155 160

Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr  
 165 170 175

15 Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
 180 185

20 <210> CO590  
 <211> 187  
 <212> PRT  
 <213> Homo sapiens

25 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
 5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
 20 25 30

30 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45

35 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60

Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80

40 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr  
 85 90 95

Ser Gly Gly Gly Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala  
 100 105 110

45 Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe  
 115 120 125

50 Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val  
 130 135 140

Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly  
 145 150 155 160

55 Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr  
 165 170 175

Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
 180 185

<210> CO589

<211> 190

5 <212> PRT

<213> Homo sapiens

Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
5 10 15

10 His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
20 25 30

15 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
50 55 60

20 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
65 70 75 80

Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly  
85 90 95

25 Ser Pro Gly Gly Gly Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile  
100 105 110

30 Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met  
115 120 125

Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys  
130 135 140

35 Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu  
145 150 155 160

Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu  
165 170 175

40 Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
180 185

45 <210> CO588

<211> 190

<212> PRT

<213> Homo sapiens

50 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
5 10 15

His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe  
20 25 30

55 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
35 40 45

Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser

	50		55		60
	Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys				
	65		70		75 80
5	Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly				
		85		90	95
10	Ser Pro Pro Gly Gly Pro Pro Gly Ser Ile Lys Glu Glu His Val Ile				
		100		105	110
	Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met				
		115		120	125
15	Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys				
		130		135	140
	Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu				
20			150	155	160
	Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu				
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25	Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn				
		180		185	
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	<211> 194				
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		20		25	30
40	Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr				
		35		40	45
	Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser				
		50		55	60
45	Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys				
		65		70	75 80
50	Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly				
		85		90	95
	Ser Pro Gly Gly Gly Gly Ser Gly Gly Gly Pro Gly Ser Ile Lys Glu				
		100		105	110
55	Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser				
		115		120	125
	Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp				
		130		135	140

Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe  
 145 150 155 160  
 5 Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys  
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 10 Asn  
 <210> CO586  
 15 <211> 194  
 <212> PRT  
 <213> Homo sapiens  
 20 Met Gly Asp Thr Arg Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys  
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 25 Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr  
 35 40 45  
 Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser  
 50 55 60  
 30 Gln Lys Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys  
 65 70 75 80  
 35 Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr  
 85 90 95  
 Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Ile Lys Glu  
 100 105 110  
 40 Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser  
 115 120 125  
 Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp  
 130 135 140  
 45 Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe  
 145 150 155 160  
 50 Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys  
 165 170 175  
 Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr  
 180 185 190  
 55 Asn

<211> 639  
 <212> DNA  
 <213> Homo sapiens

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10 gccgcggtgg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcctg 360
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   catgtggata tggcaaagaa ggagacggtc tggcggcttg aagaatttgg acgatttgcc 540
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15 atgacaaaagc gctccaacta tactccgatc accaattaa 639

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<210> C0597

<211> 639

<212> DNA

20 <213> Homo sapiens

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   catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
25  caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
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   gccgcggtgg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360
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<210> C0596

35 <211> 636

<212> DNA

<213> Homo sapiens

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   catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
   caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
   ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
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45  cggcgaggag gtggaggcag catcaaagaa gaacatgtga tcatccaggc cgagttctat 420
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   gtggatatgg caaagaagga gacggtcttg cggcttgaag aatttgagc atttgccagc 540
   tttgaggctc aaggtgcatt ggccaacata gctgtggaca aagccaacct ggaaatcatg 600
   acaaagcgct ccaactatac tccgatcacc aattaa 636
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<210> C0595

<211> 633

<212> DNA

55 <213> Homo sapiens

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   catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
   caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240

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cggcgaggag gtggaggcat caaagaagaa catgtgatca tccaggccga gttctatctg 420  
5 aatcctgacc aatcaggcga gtttatgttt gactttgatg gtgatgagat ttccatgtg 480  
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<210> CO593

10 <211> 642

<212> DNA

<213> Homo sapiens

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gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600  
25 atcatgacaa agcgcctcaa ctatactccg atcaccaatt aa 642

<210> CO585

<211> 645

<212> DNA

<213> Homo sapiens

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catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
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gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
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40 ttgtccagct ttgaggctca aggtgcattg gccaacatag ctgtggacaa agccaacctg 600  
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<210> CO584

<211> 642

45 <212> DNA

<213> Homo sapiens

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catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180  
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gccgcggttg acacctactg cagacacaac tacgggggttg gtgagagctt cacagtgcag 360  
cggcgaaagt gcggtagtgg cggtagtatc aaagaagaac atgtgatcat ccaggccgag 420  
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ttccatgtgg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 540  
gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaagc caacctggaa 600  
atcatgacaa agcgcctcaa ctatactccg atcaccaatt aa 642



&lt;210&gt; CO583

&lt;211&gt; 642

&lt;212&gt; DNA

5 &lt;213&gt; Homo sapiens

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catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
10 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
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15 ttctatctga atcctgacca atcaggcgag tttatgtttg actttgatgg tgatgagatt 480
ttccatgttg atatggcaaa gaaggagacg gtctggcggc ttgaagaatt tggacgattt 540
gccagctttg aggctcaagg tgcattggcc aacatagctg tggacaaaagc caacctggaa 600
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&lt;210&gt; CO582

20 &lt;211&gt; 651

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

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ggagggggcg gaagcggcgg aggggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
25 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggttg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360
30 cggcgaggct ctctggagg tggaggctct ggatctatca aagaagaaca tgtgatcatc 420
caggccgagt tctatctgaa tcctgaccaa tcaggcgagt ttatgtttga ctttgatggt 480
gatgagattt tccatgtgga tatggcaaag aaggagacgg tctggcggct tgaagaattt 540
ggacgatttg ccagctttga ggctcaagggt gcattggcca acatagctgt ggacaaagcc 600
35 aacctggaaa tcatgacaaa gcgctccaac tatactccga tcaccaatta a 651

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&lt;210&gt; CO581

&lt;211&gt; 651

&lt;212&gt; DNA

40 &lt;213&gt; Homo sapiens

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ggagggggcg gaagcggcgg aggggggggac acccgaccac gtttcttgga gcaggttaaa 120
catgagtgtc atttcttcaa cgggacggag cgggtgcggt tcctggacag atacttctat 180
45 caccaagagg agtacgtgcg cttcgacagc gacgtggggg agtaccgggc ggtgacggag 240
ctggggcggc ctgatgccga gtactggaac agccagaagg acctcctgga gcagaagcgg 300
gccgcggttg acacctactg cagacacaac tacgggggtg gtgagagctt cacagtgcag 360
cggcgaggct ctctcctggt tggaccacct ggatctatca aagaagaaca tgtgatcatc 420
caggccgagt tctatctgaa tcctgaccaa tcaggcgagt ttatgtttga ctttgatggt 480
50 gatgagattt tccatgtgga tatggcaaag aaggagacgg tctggcggct tgaagaattt 540
ggacgatttg ccagctttga ggctcaagggt gcattggcca acatagctgt ggacaaagcc 600
aacctggaaa tcatgacaaa gcgctccaac tatactccga tcaccaatta a 651

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55 &lt;210&gt; CO580

&lt;211&gt; 663

&lt;212&gt; DNA

&lt;213&gt; Homo sapiens

5	atgggggaca	cgggtcgcag	cttcacctg	gcctccagcg	agaccggcgt	gggcgctagc	60
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	catgagtgtc	atttcttcaa	cgggacggag	cgggtgcggt	tcctggacag	atacttctat	180
	caccaagagg	agtacgtgcg	cttcgacagc	gacgtggggg	agtaccgggc	ggtgacggag	240
	ctggggcggc	ctgatgccga	gtactggaac	agccagaagg	acctcctgga	gcagaagcgg	300
	gccgcggttg	acacctactg	cagacaacaac	tacgggggtg	gtgagagctt	cacagtgcag	360
	cggcgagggg	gtccaggcgg	tggcggggagc	ggcggtggtc	ctgggagtat	caaagaagaa	420
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10	gactttgatg	gtgatgagat	tttccatgtg	gatatggcaa	agaaggagac	ggtctggcgg	540
	cttgaagaat	ttggacgatt	tgccagcttt	gaggctcaag	gtgcattggc	caacatagct	600
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	taa						663

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15    <210> C0567
      <211> 663
      <212> DNA
      <213> Homo sapiens
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	catgagtgtc	atttcttcaa	cgggacggag	cgggtgcggt	tcctggacag	atacttctat	180
	caccaagagg	agtacgtgcg	cttcgacagc	gacgtggggg	agtaaccgggc	ggtgacggag	240
	ctggggcggc	ctgatgccga	gtactggaac	agccagaagg	acctcctgga	gcagaagcgg	300
25	gccgcggtgg	acacctactg	cagacacaac	tacgggggtg	gtgagagctt	cacagtgcag	360
	cggcgaacta	gtggtggcgg	tggcagcggc	ggtggtggtt	cctcgagtat	caaagaagaa	420
	catgtgatca	tccaggccga	gttctatctg	aatcctgacc	aatcaggcga	gtttatgttt	480
	gactttgatg	gtgatgagat	tttccatgtg	gatatggcaa	agaaggagac	ggtctggcgg	540
	ctgaagaat	ttggacgatt	tgccagcttt	gaggctcaag	gtgcattggc	caacatagct	600
30	gtggacaaag	ccaacctgga	aatcatgaca	aagcgtccca	actatactcc	gatcaccaat	660
	taa						663

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35      <210> C0598
        <211> 213
        <212> PRT
        <213> Homo sapiens

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40 Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
20 25 30

45    Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
                35                            40                            45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
50 55 60

50 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
85                      90                      95

55

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
100 105 110

Val Gly Glu Ser Phe Thr Val Leu Arg Arg Leu Gly Gly Glu Asp Asp  
 115 120 125  
 5 Glu Ala Asp His His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro  
 130 135 140  
 Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe  
 145 150 155 160  
 10 His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe  
 165 170 175  
 Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala  
 180 185 190  
 15 Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr  
 195 200 205  
 20 Pro Ile Thr Asn  
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 30 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
 20 25 30  
 35 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
 35 40 45  
 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
 50 55 60  
 40 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
 65 70 75 80  
 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
 85 90 95  
 45 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
 100 105 110  
 50 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Ser Gly Gly  
 115 120 125  
 Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro  
 130 135 140  
 55 Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe  
 145 150 155 160  
 His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe  
 165 170 175

Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala  
 180 185 190

5 Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr  
 195 200 205

Pro Ile Thr Asn  
 210

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<210> CO596  
 <211> 212  
 <212> PRT

15 <213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
 5 10 15

20 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
 20 25 30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
 35 40 45

25 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
 50 55 60

30 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
 65 70 75 80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
 85 90 95

35 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
 100 105 110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Gly Gly Gly Ser Ile  
 115 120 125

40 Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn Pro Asp  
 130 135 140

45 Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile Phe His  
 145 150 155 160

Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu Phe Gly  
 165 170 175

50 Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile Ala Val  
 180 185 190

Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr Thr Pro  
 195 200 205

55 Ile Thr Asn  
 210

5

10

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40

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50

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Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
20 25 30

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<210> C0585
<211> 215
<212> PRT
<213> Homo sapiens
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Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
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5 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Phe Asp Ala Pro Ser Pro  
                     115                    120                    125

Leu Pro Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu  
                     130                    135                    140

10 Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu  
                     145                    150                    155                    160

15 Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu  
                     165                    170                    175

Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn  
                     180                    185                    190

20 Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn  
                     195                    200                    205

Tyr Thr Pro Ile Thr Asn  
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 <212> PRT

30 <213> Homo sapiens

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35 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
                     20                    25                    30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
                     35                    40                    45

40 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
                     50                    55                    60

45 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
                     65                    70                    75                    80

Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
                     85                    90                    95

50 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
                     100                    105                    110

Val Gly Glu Ser Phe Thr Val Gln Arg Arg Ser Gly Gly Ser Gly Gly  
                     115                    120                    125

55 Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn  
                     130                    135                    140

Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile

145                      150                      155                      160  
 Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu  
                                  165                      170                      175  
 5 Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile  
                                  180                      185                      190  
 10 Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr  
                                  195                      200                      205  
 Thr Pro Ile Thr Asn  
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 15 <210> C0583  
     <211> 214  
     <212> PRT  
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 20 Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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 25 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
                                  20                      25                      30  
 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
                                  35                      40                      45  
 30 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
                                  50                      55                      60  
 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
                                  65                      70                      75                      80  
 35 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
                                  85                      90                      95  
 40 Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
                                  100                      105                      110  
 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr Ser Gly Gly Gly Gly  
                                  115                      120                      125  
 45 Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr Leu Asn  
                                  130                      135                      140  
 Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp Glu Ile  
                                  145                      150                      155                      160  
 50 Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu Glu Glu  
                                  165                      170                      175  
 Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala Asn Ile  
                                  180                      185                      190  
 55 Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser Asn Tyr  
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Thr Pro Ile Thr Asn  
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5 <210> C0582  
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<212> PRT  
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10 Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
20 25 30

15 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
35 40 45

20 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
50 55 60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
65 70 75 80

25 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
85 90 95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
100 105 110

30 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Gly Gly Gly  
115 120 125

35 Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe  
130 135 140

Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly  
145 150 155 160

40 Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg  
165 170 175

Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu  
180 185 190

45 Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg  
195 200 205

50 Ser Asn Tyr Thr Pro Ile Thr Asn  
210 215

55 <210> C0581  
<211> 217  
<212> PRT  
<213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
5 10 15

Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
                   20                  25                  30

5 Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
           35                  40                  45

Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
       50                  55                  60

10 Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu  
       65                  70                  75                  80

15 Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu  
                   85                  90                  95

Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly  
                   100                  105                  110

20 Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Pro Gly Gly  
           115                  120                  125

Pro Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe  
       130                  135                  140

25 Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly  
       145                  150                  155                  160

30 Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg  
                   165                  170                  175

Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu  
           180                  185                  190

35 Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg  
           195                  200                  205

Ser Asn Tyr Thr Pro Ile Thr Asn  
       210                  215

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<210> CO580  
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45 <213> Homo sapiens

Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly  
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50 Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg  
           20                  25                  30

Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly  
           35                  40                  45

55 Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu  
       50                  55                  60

Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu

	65		70		75		80
	Leu Gly Arg Pro Asp	85	Ala Glu Tyr Trp Asn Ser Gln Lys Asp	90	Leu Leu	95	
5	Glu Gln Lys Arg Ala Ala Val Asp	100	Thr Tyr Cys Arg His Asn Tyr Gly	105	110		
	Val Gly Glu Ser Phe Thr Val Gln Arg Arg Gly Ser Pro Gly Gly Gly	115	120	125			
10	Gly Ser Gly Gly Gly Pro Gly Ser Ile Lys Glu Glu His Val Ile Ile	130	135	140			
15	Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe	145	150	155	160		
	Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu	165	170	175			
20	Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala	180	185	190			
25	Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile	195	200	205			
	Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn	210	215	220			
30	<210> C0567 <211> 221 <212> PRT <213> Homo sapiens						
35	Met Gly Asp Thr Gly Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly	5	10	15			
40	Val Gly Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Thr Arg	20	25	30			
	Pro Arg Phe Leu Glu Gln Val Lys His Glu Cys His Phe Phe Asn Gly	35	40	45			
45	Thr Glu Arg Val Arg Phe Leu Asp Arg Tyr Phe Tyr His Gln Glu Glu	50	55	60			
	Tyr Val Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu	65	70	75	80		
50	Leu Gly Arg Pro Asp Ala Glu Tyr Trp Asn Ser Gln Lys Asp Leu Leu	85	90	95			
	Glu Gln Lys Arg Ala Ala Val Asp Thr Tyr Cys Arg His Asn Tyr Gly	100	105	110			
55	Val Gly Glu Ser Phe Thr Val Gln Arg Arg Thr Ser Gly Gly Gly Gly	115	120	125			

Ser Gly Gly Gly Gly Ser Ser Ser Ile Lys Glu Glu His Val Ile Ile  
 130 135 140

5 Gln Ala Glu Phe Tyr Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe  
 145 150 155 160

Asp Phe Asp Gly Asp Glu Ile Phe His Val Asp Met Ala Lys Lys Glu  
 165 170 175

10 Thr Val Trp Arg Leu Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala  
 180 185 190

Gln Gly Ala Leu Ala Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile  
 195 200 205

15 Met Thr Lys Arg Ser Asn Tyr Thr Pro Ile Thr Asn  
 210 215 220

# I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck nucleotide sequence

20 gcggcgcgcca ccatggagac agacacactc ctgctatggg tactgctgct ctggggttcca 60  
 ggttccactg gtgacttcaa gaacattgtg acacctcgaa caccacctcc agctagcgga 120  
 gggggcgga ggcgggagg gggagactcc gaaaggcatt tcgtgttcca gttcaagggc 180  
 25 gagtgtact tcaccaacgg gacgcagcgc atacgatctg tggacagata catctacaac 240  
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 gacacgggtg gcagacacaa ctacgagggg gtggagaccc acacctccct gcggcggtt 420  
 gaacagccca atgtcgtcat ctccctgtcc aggacagagg cctcaacca ccacaacact 480  
 ctggtctgct cagtgcagaga tttctaccca gccaatgca aagtgcgctg gttccggaat 540  
 30 ggccaggagg agacgggtggg ggtctcatcc acacagctta ttaggaatgg ggactggacc 600  
 ttccaggctc tgggtcatgt ggagatgacc ctcggcgagg gagaggtcta cacctgccac 660  
 gtggagcatc cgagcctgaa gagccccatc actgtggagt ggactagtgg tggcggtggc 720  
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 40 atcacatggc tcagaaatag taagtcatgc acagacggcg tttatgagac cagcttcctt 1200  
 gtcaaccgtg accattcctt ccacaagctg tcttatctca cttcatccc tctgacgat 1260  
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 gctagcggag ggggcggaag cggcgagggg ggagctgatg ctgcaccaac tgtatccatc 1380  
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 45 aacttctacc ccaaagacat caatgtcaag tggaagattg atggcagtga acgacaaaat 1500  
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 cacaagacat caacttcacc cattgtcaag agcttcaaca ggaatgagtg ttaggggtacc 1680

# 50 I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.Ck amino acid sequence

Met Glu Thr Asp Thr Leu Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
 5 10 15

55 Gly Ser Thr Gly Asp Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro  
 20 25 30

Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser Glu Arg  
                   35                                  40                                  45

5 His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn Gly Thr  
           50                                  55                                  60

Gln Arg Ile Arg Ser Val Asp-Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr  
   65                                  70                                  75                                  80

10 Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu  
                                   85                                  90                                  95

15 Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu Gln Thr  
                                   100                                  105                                  110

Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly Val Glu  
           115                                  120                                  125

20 Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val Ile Ser  
           130                                  135                                  140

Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val Cys Ser  
   145                                  150                                  155                                  160

25 Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe Arg Asn  
                                   165                                  170                                  175

30 Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile Arg Asn  
                                   180                                  185                                  190

Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr Pro Arg  
           195                                  200                                  205

35 Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu Lys Ser  
           210                                  215                                  220

Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly  
   225                                  230                                  235                                  240

40 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Glu Asp  
                                   245                                  250                                  255

45 Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val Tyr Gln  
           260                                  265                                  270

Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu  
           275                                  280                                  285

50 Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met Leu Pro  
           290                                  295                                  300

Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu Gln Asn  
   305                                  310                                  315                                  320

55 Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg Ser Asn  
                                   325                                  330                                  335

Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe Pro Lys

340

345

350

5 Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe Val Asp  
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 Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn Ser Lys  
 370 375 380  
 10 Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn Arg Asp  
 385 390 395 400  
 His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser Asp Asp  
 405 410 415  
 15 Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu Pro Val  
 420 425 430  
 Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala  
 435 440 445  
 20 Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu  
 450 455 460  
 Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro  
 465 470 475 480  
 Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn  
 485 490 495  
 30 Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr  
 500 505 510  
 Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His  
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 Val Lys Ser Phe Asn Arg Asn Glu Cys  
 545 550

&lt;210&gt; C0528-AC

&lt;211&gt; 600

45 &lt;212&gt; DNA

&lt;213&gt; Homo sapiens

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<210> CO608-AC

<211> 642

<212> DNA

5 <213> Homo sapiens

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atcatgacaa aacgtccaa ctatactccg attaccaatt aa 642

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20 <210> CO528

<211> 600

<212> DNA

<213> Homo sapiens

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30 aactacgggg ttggtgagag cttcacagtg cagcggcgag gaggtatcaa agaagaacat 360
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tttgatggtg atgagatttt ccatgtggat atggcaaga aggagacggt ctggcggctt 480
gaagaatttg gacgatttgc cagctttgag gctcaagggt cattggccaa catagctgtg 540
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35

<210> CO608

<211> 630

<212> DNA

40 <213> Homo sapiens

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GCCGCGGTGGACACCTACTGCAGACACAACCTACGGGGTTGGTGAGAGCTTCACAGTGCAGCGGCGAACTAGT
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CGGCTTGAAGAATTTGGACGATTTGCCAGCTTTGAGGCTCAAGGTGCATTGGCCAAACATAGCTGTGGACAAA
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<210> CO 608 variation

<211> 642

55 <212> DNA--CO608

<213> Homo sapiens

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5

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55

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<210> CO608-AC
<211> 214
<212> PRT
<213> Homo sapiens
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<210> C0608

<211> 209

<212> PRT

<213> Homo sapiens

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<210> C0608-variation

<211> 211

<212> PRT

<213> Homo sapiens

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<210> C0528
<211> 200
<212> PRT
<213> Homo sapiens
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Val Gly Gly Gly Gly Ser Arg Pro Arg Phe Leu Glu Gln Val Lys His  
20 25 30

Glu Cys His Phe Phe Asn Gly Thr Glu Arg Val Arg Phe Leu Asp Arg  
35 40 45

Tyr Phe Tyr His Gln Glu Glu Tyr Val Arg Phe Asp Ser Asp Val Gly  
50 55 60

Glu Tyr Arg Ala Val Thr Glu Leu Gly Arg Pro Asp Ala Glu Tyr Trp  
65 70 75 80

Asn Ser Gln Arg Asp Leu Leu Glu Gln Lys Arg Ala Ala Val Asp Thr  
85 90 95

Tyr Cys Arg His Asn Tyr Gly Val Gly Glu Ser Phe Thr Val Gln Arg  
100 105 110

Arg Gly Gly Ile Lys Glu Glu His Val Ile Ile Gln Ala Glu Phe Tyr  
115 120 125

Leu Asn Pro Asp Gln Ser Gly Glu Phe Met Phe Asp Phe Asp Gly Asp  
130 135 140

Glu Ile Phe His Val Asp Met Ala Lys Lys Glu Thr Val Trp Arg Leu  
145 150 155 160

Glu Glu Phe Gly Arg Phe Ala Ser Phe Glu Ala Gln Gly Ala Leu Ala  
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Asn Ile Ala Val Asp Lys Ala Asn Leu Glu Ile Met Thr Lys Arg Ser  
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Asn Tyr Thr Pro Ile Thr Asn  
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<210> IAS MBP1-14 CH1.CH2.CH3
<211> 2346
<212> DNA
<213> murine
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	cggcttgaac	agcccaatgt	cgtcatctcc	ctgtccagga	cagaggccct	caaccaccac	480
	aacactctgg	tctgctcagt	gacagatttc	taccagcca	agatcaaagt	gcgctgggtc	540
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40 <210> IAS MBP 1-14 CH1.H  
 <211> 1701  
 <212> DNA  
 <213> murine

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<210> IAS MBP 90-101 CH1.H.CH2

<211> 2053

<212> DNA

20 <213> murine

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<210> IAS MBP 90-101 CH1.H

<211> 1707  
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   agctcagtga ctgtcccctc cagcacctgg cccagcgaga ccgtcacctg caacgttgcc 1620
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35  
 <210> MBP 1-14 CK  
 <211> 1686  
 <212> DNA  
 <213> murine

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   agcggagggg gcggaagcgg cggaggggga gactccgaaa ggcatttcgt gttccagttc 180
   aagggcgagt gctacttcac caacgggacg cagcgcatat gatctgtgga cagatacatc 240
45 tacaaccggg aggagtacct gcgcttcgac agcgacgtgg gcgagtaccg cgcggtgacc 300
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   cggttgaac agcccaatgt cgtcatctcc ctgtccagga cagaggccct caaccaccac 480
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   gtgctgctgg gtcagcccaa caccctcatc tgctttgtgg acaacatctt ccctcctgtg 1140

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 ttccttgatca accgtgacca ttccttccac aagctgtctt atctcacctt catcccttct 1260  
 gacgatgata tttatgactg caaggtggag cactggggcc tggaggagcc gggtctgaaa 1320  
 5 cactgggcta gcggaggggg cggaagcggc ggagggggag ctgatgctgc accaactgta 1380  
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<210> MBP 1-14 CH1.H.CH2

<211> 2059

15 <212> DNA

<213> murine

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 aagggcgagt gctacttcac caacgggacg cagcgcatat gatctgtgga cagatacatc 240  
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 25 gagctggaca cgggtgtgcag acacaactac gaggggggtgg agaccacac ctccctgcgg 420  
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 35 tcaaattcca cccagctac caatgaggct cctcaagcga ctgtgttccc caagtcccct 1080  
 gtgctgctgg gtcagcccaa caccctcatc tgctttgtgg acaacatctt cctcctgtg 1140  
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 50 gtgaacaacg tggaagtaca cacagctcag acacaaacc atagagagga ttacaacagt 1920  
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55 <210> MBP 90-101 CH1.H.CH2.CH3

<211> 2343

<212> DNA--

<213> murine

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	65							70				75				80	
5	Glu	Tyr	Leu	Arg	Phe	Asp	Ser	Asp	Val	Gly	Glu	Tyr	Arg	Ala	Val	Thr	
					85					90					95		
	Glu	Leu	Gly	Arg	Pro	Asp	Pro	Glu	Tyr	Tyr	Asn	Lys	Gln	Tyr	Leu	Glu	
				100					105					110			
10	Gln	Thr	Arg	Ala	Glu	Leu	Asp	Thr	Val	Cys	Arg	His	Asn	Tyr	Glu	Gly	
			115					120					125				
	Val	Glu	Thr	His	Thr	Ser	Leu	Arg	Arg	Leu	Glu	Gln	Pro	Asn	Val	Val	
	130							135				140					
15	Ile	Ser	Leu	Ser	Arg	Thr	Glu	Ala	Leu	Asn	His	His	Asn	Thr	Leu	Val	
	145					150					155					160	
20	Cys	Ser	Val	Thr	Asp	Phe	Tyr	Pro	Ala	Lys	Ile	Lys	Val	Arg	Trp	Phe	
					165					170					175		
	Arg	Asn	Gly	Gln	Glu	Glu	Thr	Val	Gly	Val	Ser	Ser	Thr	Gln	Leu	Ile	
				180					185					190			
25	Arg	Asn	Gly	Asp	Trp	Thr	Phe	Gln	Val	Leu	Val	Met	Leu	Glu	Met	Thr	
			195					200					205				
	Pro	Arg	Arg	Gly	Glu	Val	Tyr	Thr	Cys	His	Val	Glu	His	Pro	Ser	Leu	
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30	Lys	Ser	Pro	Ile	Thr	Val	Glu	Trp	Thr	Ser	Gly	Gly	Gly	Gly	Ser	Gly	
	225					230					235					240	
	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser	Ser	
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	Glu	Asp	Asp	Ile	Glu	Ala	Asp	His	Val	Gly	Val	Tyr	Gly	Thr	Thr	Val	
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40	Tyr	Gln	Ser	Pro	Gly	Asp	Ile	Gly	Gln	Tyr	Thr	His	Glu	Phe	Asp	Gly	
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	Asp	Glu	Trp	Phe	Tyr	Val	Asp	Leu	Asp	Lys	Lys	Glu	Thr	Ile	Trp	Met	
	290						295					300					
45	Leu	Pro	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	Gln	Gly	Gly	Leu	
	305					310					315					320	
	Gln	Asn	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	Leu	Thr	Lys	Arg	
50					325					330					335		
	Ser	Asn	Ser	Thr	Pro	Ala	Thr	Asn	Glu	Ala	Pro	Gln	Ala	Thr	Val	Phe	
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55	Pro	Lys	Ser	Pro	Val	Leu	Leu	Gly	Gln	Pro	Asn	Thr	Leu	Ile	Cys	Phe	
		355						360					365				
	Val	Asp	Asn	Ile	Phe	Pro	Pro	Val	Ile	Asn	Ile	Thr	Trp	Leu	Arg	Asn	
	370							375					380				



	Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn	
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5	Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser	
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10	Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu	
		420 425 430
	Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly	
		435 440 445
15	Gly Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Cys	
		450 455 460
	Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly	
		465 470 475 480
20	Tyr Phe Pro Glu Ser Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser	
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25	Ser Ser Val His Thr Phe Pro Ala Leu Leu Gln Ser Gly Leu Tyr Thr	
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	Met Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr	
		515 520 525
30	Val Thr Cys Ser Val Ala His Pro Ala Ser Ser Thr Thr Val Asp Lys	
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	Lys Leu Glu Pro Ser Gly Pro Ile Ser Thr Ile Asn Pro Cys Pro Pro	
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35	Cys Lys Glu Cys His Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly Pro	
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40	Ser Val Phe Ile Phe Pro Pro Asn Ile Lys Asp Val Leu Met Ile Ser	
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	Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp	
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	Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Ile Arg Val	
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50	Val Ser Thr Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu	
		645 650 655
55	Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ser Pro Ile Glu Arg	
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	Thr Ile Ser Lys Ile Lys	
		675

&lt;210&gt; IAS MBP 1-14 CH1.H

&lt;211&gt; 677

&lt;212&gt; PRT--

&lt;213&gt; murine

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10 Gly Ser Thr Gly Asp Phe Lys Asn Ile Val Thr Pro Arg Thr Pro Pro  
20 25 30

Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser Glu Arg  
35 40 45

15 His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn Gly Thr  
50 55 60

Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr  
65 70 75 80

20 Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu  
85 90 95

25 Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu Gln Thr  
100 105 110

Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly Val Glu  
115 120 125

30 Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val Ile Ser  
130 135 140

Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val Cys Ser  
145 150 155 160

35 Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe Arg Asn  
165 170 175

40 Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile Arg Asn  
180 185 190

Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr Pro Arg  
195 200 205

45 Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu Lys Ser  
210 215 220

Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly Gly Gly  
225 230 235 240

50 Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser Glu Asp  
245 250 255

Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val Tyr Gln  
260 265 270

55 Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly Asp Glu  
275 280 285

Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met Leu Pro  
 290 295 300

5 Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu Gln Asn  
 305 310 315 320

Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg Ser Asn  
 325 330 335

10 Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe Pro Lys  
 340 345 350

Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe Val Asp  
 355 360 365

15 Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn Ser Lys  
 370 375 380

20 Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn Arg Asp  
 385 390 395 400

His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser Asp Asp  
 405 410 415

25 Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu Pro Val  
 420 425 430

Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ala  
 435 440 445

30 Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Cys Gly Asp  
 450 455 460

35 Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe  
 465 470 475 480

Pro Glu Ser Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Ser  
 485 490 495

40 Val His Thr Phe Pro Ala Leu Leu Gln Ser Gly Leu Tyr Thr Met Ser  
 500 505 510

Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr Val Thr  
 515 520 525

45 Cys Ser Val Ala His Pro Ala Ser Ser Thr Thr Val Asp Lys Lys Leu  
 530 535 540

50 Glu Pro Ser Gly Pro Ile Ser Thr Ile Asn Pro Cys Pro Pro Cys Lys  
 545 550 555 560

Glu Cys His Lys Cys Pro Ala Pro Asn Leu Glu Gly Gly Pro Ser Val  
 565 570 575

55 Phe Ile Phe Pro Pro Asn Ile Lys Asp Val Leu Met Ile Ser Leu Thr  
 580 585 590

Pro Lys Val Thr Cys Val Val Val Asp Val Ser Glu Asp Asp Pro Asp  
 595 600 605

Val Gln Ile Ser Trp Phe Val Asn Asn Val Glu Val His Thr Ala Gln  
 610 615 620  
 5 Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Ile Arg Val Val Ser  
 625 630 635 640  
 Thr Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly Lys Glu Phe Lys  
 645 650 655  
 10 Cys Lys Val Asn Asn Lys Asp Leu Pro Ser Pro Ile Glu Arg Thr Ile  
 660 665 670  
 Ser Lys Ile Lys  
 15 675  
 <210> IAS MBP 90-101 CH1.H.CH2  
 <211> 563  
 20 <212> PRT--  
 <213> murine  
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 30 Pro Ala Ser Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Asp Ser  
 35 40 45  
 Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn  
 50 55 60  
 35 Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu  
 65 70 75 80  
 Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr  
 85 90 95  
 40 Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu  
 100 105 110  
 Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly  
 115 120 125  
 45 Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val  
 130 135 140  
 50 Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val  
 145 150 155 160  
 Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe  
 165 170 175  
 55 Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile  
 180 185 190  
 Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr

	195	200	205
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	210	215	220
5	Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly		
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	Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser		
10		245	250 255
	Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val		
	260	265	270
15	Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly		
	275	280	285
	Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met		
20	290	295	300
	Leu Pro Glu Phe Gly Gln Leu Thr Ser Leu Ser Phe Asp Pro Gln Gly		
	305	310	315 320
25	Gly Leu Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr		
	325	330	335
	Lys Arg Ser Asn Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr		
	340	345	350
30	Val Phe Pro Lys Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile		
	355	360	365
	Cys Phe Val Asp Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu		
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	Arg Asn Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu		
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40	Val Asn Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile		
	405	410	415
	Pro Ser Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu		
	420	425	430
45	Glu Glu Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly		
	435	440	445
	Gly Gly Gly Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro		
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	Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val		
	465	470	475 480
55	Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser		
	485	490	495
	Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu		
	500	505	510

Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser  
 515 520 525

5 Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val  
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Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys  
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10 Thr Val

15 <210> IAS MBP 90-101 CH1.H  
 <211> 556  
 <212> PRT  
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25 Gly Ser Lys Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser  
 35 40 45

Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn  
 50 55 60

30 Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu  
 65 70 75 80

35 Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr  
 85 90 95

Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu  
 100 105 110

40 Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu Gly  
 115 120 125

Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val Val  
 130 135 140

45 Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu Val  
 145 150 155 160

50 Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp Phe  
 165 170 175

Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu Ile  
 180 185 190

55 Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met Thr  
 195 200 205

Pro Arg Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser Leu  
 210 215 220

Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser Gly  
 225 230 235 240  
 5 Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser Ser  
 245 250 255  
 Glu Asp Asp Ile Glu Ala Asp His Val Gly Val Tyr Gly Thr Thr Val  
 260 265 270  
 10 Tyr Gln Ser Pro Gly Asp Ile Gly Gln Tyr Thr His Glu Phe Asp Gly  
 275 280 285  
 Asp Glu Trp Phe Tyr Val Asp Leu Asp Lys Lys Glu Thr Ile Trp Met  
 290 295 300  
 15 Leu Pro Glu Phe Gly Gln Leu Thr Ser Phe Asp Pro Gln Gly Gly Leu  
 305 310 315 320  
 20 Gln Asn Ile Ala Thr Gly Lys Tyr Thr Leu Gly Ile Leu Thr Lys Arg  
 325 330 335  
 Ser Asn Ser Thr Pro Ala Thr Asn Glu Ala Pro Gln Ala Thr Val Phe  
 340 345 350  
 25 Pro Lys Ser Pro Val Leu Leu Gly Gln Pro Asn Thr Leu Ile Cys Phe  
 355 360 365  
 30 Val Asp Asn Ile Phe Pro Pro Val Ile Asn Ile Thr Trp Leu Arg Asn  
 370 375 380  
 Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn  
 385 390 395 400  
 35 Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser  
 405 410 415  
 Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu  
 420 425 430  
 40 Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly  
 435 440 445  
 45 Gly Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu  
 450 455 460  
 Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe  
 465 470 475 480  
 50 Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg  
 485 490 495  
 Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser  
 500 505 510  
 55 Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu  
 515 520 525  
 Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr Ser Ser

530

535

540

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys  
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5

<210> IAS MBP 1-14 CK

<211> 775

<212> PRT

10 <213> murine

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His Gly Ser Lys Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp  
 35 40 45

20 Ser Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr  
 50 55 60

25 Asn Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg  
 65 70 75 80

Glu Glu Tyr Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val  
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30 Thr Glu Leu Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu  
 100 105 110

Glu Gln Thr Arg Ala Glu Leu Asp Thr Val Cys Arg His Asn Tyr Glu  
 115 120 125

35 Gly Val Glu Thr His Thr Ser Leu Arg Arg Leu Glu Gln Pro Asn Val  
 130 135 140

40 Val Ile Ser Leu Ser Arg Thr Glu Ala Leu Asn His His Asn Thr Leu  
 145 150 155 160

Val Cys Ser Val Thr Asp Phe Tyr Pro Ala Lys Ile Lys Val Arg Trp  
 165 170 175

45 Phe Arg Asn Gly Gln Glu Glu Thr Val Gly Val Ser Ser Thr Gln Leu  
 180 185 190

Ile Arg Asn Gly Asp Trp Thr Phe Gln Val Leu Val Met Leu Glu Met  
 195 200 205

50 Thr Pro Arg Arg Gly Glu Val Tyr Thr Cys His Val Glu His Pro Ser  
 210 215 220

55 Leu Lys Ser Pro Ile Thr Val Glu Trp Thr Ser Gly Gly Gly Gly Ser  
 225 230 235 240

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Ser  
 245 250 255



	Ser	Glu	Asp	Asp	Ile	Glu	Ala	Asp	His	Val	Gly	Val	Tyr	Gly	Thr	Thr	
				260					265								270
5	Val	Tyr	Gln	Ser	Pro	Gly	Asp	Ile	Gly	Gln	Tyr	Thr	His	Glu	Phe	Asp	
			275					280					285				
	Gly	Asp	Glu	Trp	Phe	Tyr	Val	Asp	Leu	Asp	Lys	Lys	Glu	Thr	Ile	Trp	
			290				295					300					
10	Met	Leu	Pro	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	Gln	Gly	Gly	
	305					310					315					320	
	Leu	Gln	Asn	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	Leu	Thr	Lys	
					325					330					335		
15	Arg	Ser	Asn	Ser	Thr	Pro	Ala	Thr	Asn	Glu	Ala	Pro	Gln	Ala	Thr	Val	
				340					345					350			
20	Phe	Pro	Lys	Ser	Pro	Val	Leu	Leu	Gly	Gln	Pro	Asn	Thr	Leu	Ile	Cys	
			355					360					365				
	Phe	Val	Asp	Asn	Ile	Phe	Pro	Pro	Val	Ile	Asn	Ile	Thr	Trp	Leu	Arg	
		370					375					380					
25	Asn	Ser	Lys	Ser	Val	Thr	Asp	Gly	Val	Tyr	Glu	Thr	Ser	Phe	Leu	Val	
	385					390					395					400	
	Asn	Arg	Asp	His	Ser	Phe	His	Lys	Leu	Ser	Tyr	Leu	Thr	Phe	Ile	Pro	
				405						410					415		
30	Ser	Asp	Asp	Asp	Ile	Tyr	Asp	Cys	Lys	Val	Glu	His	Trp	Gly	Leu	Glu	
				420					425					430			
	Glu	Pro	Val	Leu	Lys	His	Trp	Ala	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	
35			435					440					445				
	Gly	Gly	Ala	Lys	Thr	Thr	Pro	Pro	Ser	Val	Tyr	Pro	Leu	Ala	Pro	Gly	
		450					455					460					
40	Ser	Ala	Ala	Gln	Thr	Asn	Ser	Met	Val	Thr	Leu	Gly	Cys	Leu	Val	Lys	
	465					470					475					480	
	Gly	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Thr	Trp	Asn	Ser	Gly	Ser	Leu	
					485					490					495		
45	Ser	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Asp	Leu	Tyr	
				500					505					510			
50	Thr	Leu	Ser	Ser	Ser	Val	Thr	Val	Pro	Ser	Ser	Thr	Trp	Pro	Ser	Glu	
			515					520					525				
	Thr	Val	Thr	Cys	Asn	Val	Ala	His	Pro	Ala	Ser	Ser	Thr	Lys	Val	Asp	
		530					535					540					
55	Lys	Lys	Ile	Val	Pro	Arg	Asp	Cys	Gly	Cys	Lys	Pro	Cys	Ile	Cys	Thr	
	545					550					555					560	
	Val	Pro	Glu	Val	Ser	Ser	Val	Phe	Ile	Phe	Pro	Pro	Lys	Pro	Lys	Asp	
					565					570							

Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp  
 580 585 590  
 5 Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp  
 595 600 605  
 Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn  
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 10 Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp  
 625 630 635 640  
 Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro  
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 15 Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala  
 660 665 670  
 20 Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp  
 675 680 685  
 Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile  
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 25 Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn  
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 30 Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys  
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 35 40 45  
 55 Glu Arg His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn  
 50 55 60  
 Gly Thr Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu

	65				70						75					80
	Glu	Tyr	Leu	Arg	Phe	Asp	Ser	Asp	Val	Gly	Glu	Tyr	Arg	Ala	Val	Thr
					85					90					95	
5	Glu	Leu	Gly	Arg	Pro	Asp	Pro	Glu	Tyr	Tyr	Asn	Lys	Gln	Tyr	Leu	Glu
				100					105					110		
	Gln	Thr	Arg	Ala	Glu	Leu	Asp	Thr	Val	Cys	Arg	His	Asn	Tyr	Glu	Gly
10			115					120					125			
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		130					135					140				
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	145					150					155					160
	Cys	Ser	Val	Thr	Asp	Phe	Tyr	Pro	Ala	Lys	Ile	Lys	Val	Arg	Trp	Phe
					165					170					175	
20	Arg	Asn	Gly	Gln	Glu	Glu	Thr	Val	Gly	Val	Ser	Ser	Thr	Gln	Leu	Ile
				180					185					190		
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25			195					200					205			
	Pro	Arg	Arg	Gly	Glu	Val	Tyr	Thr	Cys	His	Val	Glu	His	Pro	Ser	Leu
		210					215					220				
30	Lys	Ser	Pro	Ile	Thr	Val	Glu	Trp	Thr	Ser	Gly	Gly	Gly	Gly	Ser	Gly
	225					230					235					240
	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser	Ser
				245					250					255		
35	Glu	Asp	Asp	Ile	Glu	Ala	Asp	His	Val	Gly	Val	Tyr	Gly	Thr	Thr	Val
				260				265						270		
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40			275					280					285			
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		290					295					300				
45	Leu	Pro	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	Gln	Gly	Gly	Leu
	305					310					315					320
	Gln	Asn	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	Leu	Thr	Lys	Arg
				325						330					335	
50	Ser	Asn	Ser	Thr	Pro	Ala	Thr	Asn	Glu	Ala	Pro	Gln	Ala	Thr	Val	Phe
				340					345					350		
	Pro	Lys	Ser	Pro	Val	Leu	Leu	Gly	Gln	Pro	Asn	Thr	Leu	Ile	Cys	Phe
55			355					360					365			
	Val	Asp	Asn	Ile	Phe	Pro	Pro	Val	Ile	Asn	Ile	Thr	Trp	Leu	Arg	Asn
		370					375					380				

Ser Lys Ser Val Thr Asp Gly Val Tyr Glu Thr Ser Phe Leu Val Asn  
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 5 Arg Asp His Ser Phe His Lys Leu Ser Tyr Leu Thr Phe Ile Pro Ser  
 405 410 415  
 Asp Asp Asp Ile Tyr Asp Cys Lys Val Glu His Trp Gly Leu Glu Glu  
 420 425 430  
 10 Pro Val Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly  
 435 440 445  
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 450 455 460  
 15 Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly  
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 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr  
 500 505 510  
 25 Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr  
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 45 Pro Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Asp Ser Glu Arg  
 35 40 45  
 50 His Phe Val Phe Gln Phe Lys Gly Glu Cys Tyr Phe Thr Asn Gly Thr  
 50 55 60  
 Gln Arg Ile Arg Ser Val Asp Arg Tyr Ile Tyr Asn Arg Glu Glu Tyr  
 65 70 75 80  
 55 Leu Arg Phe Asp Ser Asp Val Gly Glu Tyr Arg Ala Val Thr Glu Leu  
 85 90 95  
 Gly Arg Pro Asp Pro Glu Tyr Tyr Asn Lys Gln Tyr Leu Glu Gln Thr  
 100 105 110

	Arg	Ala	Glu	Leu	Asp	Thr	Thr	Cys	Arg	His	Asn	Tyr	Glu	Gly	Val	Glu	
		115						120					125				
5	Thr	His	Thr	Ser	Leu	Arg	Arg	Leu	Glu	Gln	Pro	Asn	Val	Val	Ile	Ser	
		130					135					140					
	Leu	Ser	Arg	Thr	Glu	Ala	Leu	Asn	His	His	Asn	Thr	Leu	Val	Cys	Ser	
	145					150					155				160		
10	Val	Thr	Asp	Phe	Tyr	Pro	Ala	Lys	Ile	Lys	Val	Arg	Trp	Phe	Arg	Asn	
				165						170					175		
	Gly	Gln	Glu	Glu	Thr	Val	Gly	Val	Ser	Ser	Thr	Gln	Leu	Ile	Arg	Asn	
15				180					185					190			
	Gly	Asp	Trp	Thr	Phe	Gln	Val	Leu	Val	Met	Leu	Glu	Met	Thr	Pro	Arg	
		195						200					205				
20	Arg	Gly	Glu	Val	Tyr	Thr	Cys	His	Val	Glu	His	Pro	Ser	Leu	Lys	Ser	
		210					215					220					
	Pro	Ile	Thr	Val	Glu	Trp	Thr	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	
	225					230					235					240	
25	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Ser	Ser	Glu	Asp	
				245					250						255		
	Asp	Ile	Glu	Ala	Asp	His	Val	Gly	Val	Tyr	Gly	Thr	Thr	Val	Tyr	Gln	
30				260					265					270			
	Ser	Pro	Gly	Asp	Ile	Gly	Gln	Tyr	Thr	His	Glu	Phe	Asp	Gly	Asp	Glu	
			275					280					285				
35	Trp	Phe	Tyr	Val	Asp	Leu	Asp	Lys	Lys	Glu	Thr	Ile	Trp	Met	Leu	Pro	
		290					295					300					
	Glu	Phe	Gly	Gln	Leu	Thr	Ser	Phe	Asp	Pro	Gln	Gly	Gly	Leu	Gln	Asn	
	305					310					315					320	
40	Ile	Ala	Thr	Gly	Lys	Tyr	Thr	Leu	Gly	Ile	Leu	Thr	Lys	Arg	Ser	Asn	
				325						330					335		
	Ser	Thr	Pro	Ala	Thr	Asn	Glu	Ala	Pro	Gln	Ala	Thr	Val	Phe	Pro	Lys	
45				340					345					350			
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			355					360					365				
50	Asn	Ile	Phe	Pro	Pro	Val	Ile	Asn	Ile	Thr	Trp	Leu	Arg	Asn	Ser	Lys	
		370					375					380					
	Ser	Val	Thr	Asp	Gly	Val	Tyr	Glu	Thr	Ser	Phe	Leu	Val	Asn	Arg	Asp	
	385					390					395					400	
55	His	Ser	Phe	His	Lys	Leu	Ser	Tyr	Leu	Thr	Phe	Ile	Pro	Ser	Asp	Asp	
				405						410					415		
	Asp	Ile	Tyr	Asp	Cys	Lys	Val	Glu	His	Trp	Gly	Leu	Glu	Glu	Pro	Val	

	420	425	430	
	Leu Lys His Trp Ala Ser Gly Gly Gly Gly Ser Gly Gly Gly Ser Leu			
	435	440	445	
5	Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala			
	450	455	460	
	Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr			
10	465	470	475	480
	Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser			
	485	490	495	
15	Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu			
	500	505	510	
	Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr Val			
20	515	520	525	
	Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys			
	530	535	540	
	Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro			
25	545	550	555	560
	Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu			
	565	570	575	
30	Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser			
	580	585	590	
	Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu			
35	595	600	605	
	Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr			
	610	615	620	
	Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu Asn			
40	625	630	635	640
	Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro			
	645	650	655	
45	Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln			
	660	665	670	
	Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val			
50	675	680	685	
	Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val			
	690	695	700	
	Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln			
55	705	710	715	720
	Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn			
	725	730	735	

111

Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val  
740 745 750

5 Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser His  
755 760 765

Ser Pro Gly Lys  
770

10

WHAT IS CLAIMED IS:

- 1                   1.       A multimeric complex comprising a first single chain MHC class II  
2 heterodimer and a second single chain MHC class II heterodimer, wherein the first and  
3 the second single chain MHC class II heterodimers each comprise an  $\alpha$ 1 domain and a  $\beta$ 1  
4 domain linked via an amino acid linker and a multimerization domain, and wherein the  
5 first and the second single chain MHC class II heterodimers are linked via the  
6 multimerization domain to form a multimeric complex.
- 1                   2.       The multimeric complex of claim 1, wherein the first and the  
2 second single chain MHC class II heterodimers are each linked to an antigenic peptide via  
3 an amino acid linker.
- 1                   3.       The multimeric complex of claim 2, wherein the peptides are the  
2 same.
- 1                   4.       The multimeric complex of claim 2, wherein the peptides are from  
2 the same antigen.
- 1                   5.       The multimeric complex of claim 2, wherein the peptides are from  
2 different antigens.
- 1                   6.       The multimeric complex of claim 2, wherein the peptides are  
2 selected from the group consisting of MBP83-102Y83, PLP 40-60, PLP 89-106, PLP 95-  
3 117, and PLP 185-206.
- 1                   7.       The multimeric complex of claim 1, wherein the first and the  
2 second MHC class II heterodimers are from the same MHC class II allele.
- 1                   8.       The multimeric complex of claim 1, wherein the first and the  
2 second MHC class II heterodimers are from different MHC class II alleles.
- 1                   9.       The multimeric complex of claim 7 or 8, wherein the alleles are  
2 selected from the group consisting of DRB1\*1501 and DRB5\*0101.



1                   10.    The multimeric complex of claim 7 or 8, wherein the first and the  
2   second single chain MHC class II heterodimers are each linked to an antigenic peptide via  
3   an amino acid linker.

1                   11.    The multimeric complex of claim 10, wherein the peptides are the  
2   same.

1                   12.    The multimeric complex of claim 10, wherein the peptides are  
2   from the same antigen.

1                   13.    The multimeric complex of claim 10, wherein the peptides are  
2   from different antigens.

1                   14.    The multimeric complex of claim 10, wherein the peptides are  
2   selected from the group consisting of MBP83-102Y83, PLP 40-60, PLP 89-106, PLP 95-  
3   117, and PLP 185-206.

1                   15.    The multimeric complex of claim 1, wherein the first and second  
2   MHC class II heterodimers are from a human.

1                   16.    The multimeric complex of claim 1, wherein the multimerization  
2   domains are covalently linked.

1                   17.    The multimeric complex of claim 1, wherein the multimerization  
2   domains are non-covalently linked.

1                   18.    The multimeric complex of claim 1, wherein the multimerization  
2   domain is a leucine zipper domain.

1                   19.    The multimeric complex of claim 1, wherein the multimerization  
2   domain is an immunoglobulin domain.

1                   20.    The multimeric complex of claim 19, wherein the immunoglobulin  
2   domain is a C $\kappa$  domain or a heavy chain domain.

1                   21.     The multimeric complex of claim 1, wherein the first or the second  
2 MHC class II heterodimer is selected from the group consisting of I-As  
3 MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.C $\kappa$ , I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CH1.H, I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CH1.H.CH2,  
4 and I-As MBP. $\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.CH1.H.CH2.CH3.

1                   22.     The multimeric complex of claim 1, further comprising a third and  
2 a fourth MHC class II heterodimer, wherein the third and the fourth MHC class II  
3 heterodimer each comprise an  $\alpha$ 1 domain and a  $\beta$ 1 domain linked via an amino acid linker  
4 and a multimerization domain, and wherein the first, second, third and fourth single chain  
5 MHC class II heterodimers are linked via the multimerization domain to form a  
6 multimeric complex.

1                   23.     The multimeric complex of claim 1, wherein the first and the  
2 second MHC class II heterodimers comprise  $\beta$ 1  $\beta$ 2 domains and  $\alpha$ 1  $\alpha$ 2 domains linked via  
3 an amino acid linker.

1                   24.     The multimeric complex of claim 1 or 2, wherein the linker is  
2 selected from the group consisting of GG, GGGG, GGGGS, GGGSG, ASGGGS, GGGG,  
3 TSGGGGS, GGGGSSS, GSPGGGGSGGGPGS, GSPPGGPPGS, GSPGGGGPGS,  
4 TSGGGGS, SGGSGGS, FDAPSPLP, and VYPEVTV.

1                   25.     The multimeric complex of claim 1 or 2, wherein the linker is from  
2 a CD4 molecule.

1                   26.     The multimeric complex of claim 25, wherein the linker is selected  
2 from the group consisting of RNGQEEKAGVVSTGLI, RNGQETKAGVVSTGLI,  
3 YNQEEKAGGVSTGLI, FRNGQEEKAGVVSTGLI, FRNGQETKAGVVSTGLI,  
4 FYNQEEKAGGVSTGLI, and LNGQEEKAGMVSTGLI.

1                   27.     The multimeric complex of claim 1, wherein the first or the second  
2 MHC class II heterodimer is selected from the group consisting of CO523, CO543,  
3 CO563, CO567, CO580, CO581, CO582, CO583, CO584, CO585, CO586, CO587,  
4 CO588, CO589, CO590, CO591, CO592, CO593, CO594, CO595, CO596, CO597, and  
5 CO608.

1                   28.    The multimeric complex of claim 1, wherein the first MHC class II  
2    or the second MHC class II heterodimer is encoded by a nucleic acid that codons  
3    optimized for prokaryotic expression.

1                   29.    The multimeric complex of claim 28, wherein the prokaryote is *E.*  
2    *coli*.

1                   30.    The multimeric complex of claim 28, wherein the nucleic acid  
2    encoding the first or the second MHC class II heterodimer is selected from the group  
3    consisting of CO528-AC and CO608-AC.

1                   31.    A pharmaceutical composition comprising the multimeric complex  
2    of claim 2.

1                   32.    The pharmaceutical composition of claim 31, further comprising an  
2    adjuvant.

1                   33.    A method of treating autoimmune disease in a subject, the method  
2    comprising administering an immunogenically effective amount of a pharmaceutical  
3    composition comprising the multimeric complex of claim 2.

1                   34.    The method of claim 33, wherein the subject is a human.

1                   35.    A recombinant nucleic acid encoding a single chain MHC class II  
2    heterodimer comprising an  $\alpha$ 1 domain and a  $\beta$ 1 domain linked via an amino acid linker,  
3    wherein the linker is selected from the group consisting of GG, GGGG, GGGGS,  
4    GGGSG, ASGGGSGGG, TSGGGGSGGGGSSS, GSPGGGGSGGGPGS,  
5    GSPPGGPPGS, GSPGGGGPGS, TSGGGGS, SGGSGGS, FDAPSPLP, and VYPEVTV.

1                   36.    The nucleic acid of claim 35, wherein the MHC class II  
2    heterodimer is selected from the group consisting of CO523, CO543, CO563, CO567,  
3    CO580, CO581, CO582, CO583, CO584, CO585, CO586, CO587, CO588, CO589,  
4    CO590, CO591, CO592, CO593, CO594, CO595, CO596, CO597, and CO608.

1                    37.    A recombinant nucleic acid encoding a single chain MHC class II  
2 heterodimer comprising an  $\alpha 1$  domain and a  $\beta 1$  domain linked via an amino acid linker,  
3 wherein the linker is wherein the linker is from a CD4 molecule.

1                    38.    The nucleic acid of claim 37, wherein the linker is selected from  
2 the group consisting of RNGQEEKAGVVSTGLI, RNGQETKAGVVSTGLI,  
3 YNQEEKAGGVSTGLI, FRNGQEEKAGVVSTGLI, FRNGQETKAGVVSTGLI,  
4 FYNQEEKAGGVSTGLI, and LNGQEEKAGMVSTGLI.

1                    39.    A recombinant nucleic acid encoding a single chain MHC class II  
2 heterodimer selected from the group consisting of CO528-AC and CO608-AC.

1                    40.    A recombinant nucleic acid encoding a single chain MHC class II  
2 heterodimer selected from the group consisting I-As MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .C $\kappa$ , I-As  
3 MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .CH1.H, I-As MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .CH1.H.CH2, and I-As  
4 MBP. $\beta 1\beta 2\alpha 1\alpha 2$ .CH1.H.CH2.CH3.

**IMMUNE MEDIATORS AND RELATED METHODS****ABSTRACT OF THE INVENTION**

The present invention relates to nucleic acids encoding single chain MHC  
5 class II molecules that form multimers via inter-chain multimerization domains, and  
methods of treating autoimmune disease using the same.

10

SF 1139077 v1

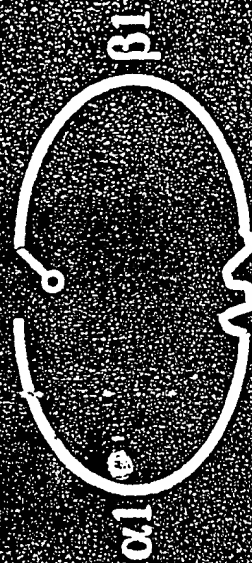


mIgGyl

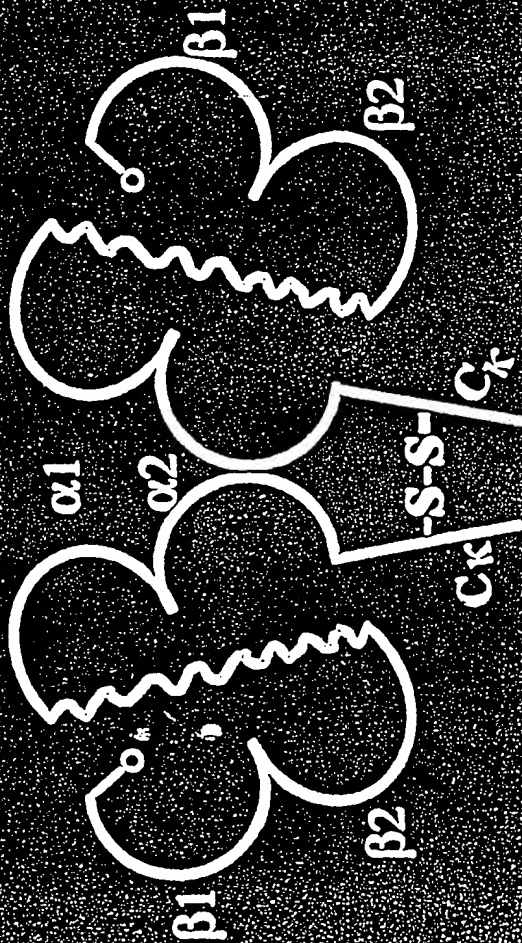


I-A's MBP.β1β2α1α2.Cκ

I-A's MBP.β1α1



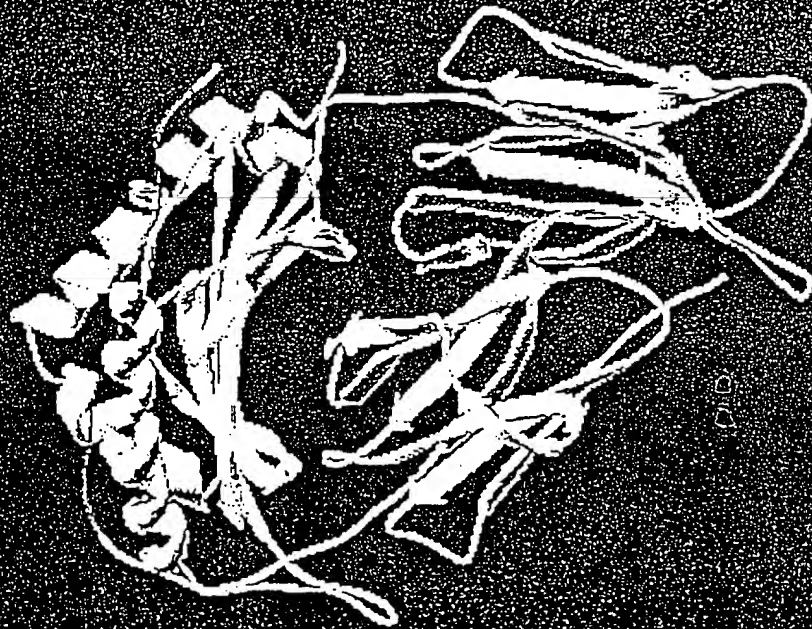
Monomer



Dimer

Figure 1

**I-E $\kappa$ :HSP70**



**I-A $\kappa$ MBP $\beta$ 1 $\alpha$ 1**  
**(Monomer)**



**I-A $\kappa$ MBP**  
 **$\beta$ 1 $\beta$ 2 $\alpha$ 1 $\alpha$ 2.C $\kappa$**   
**(Dimer)**

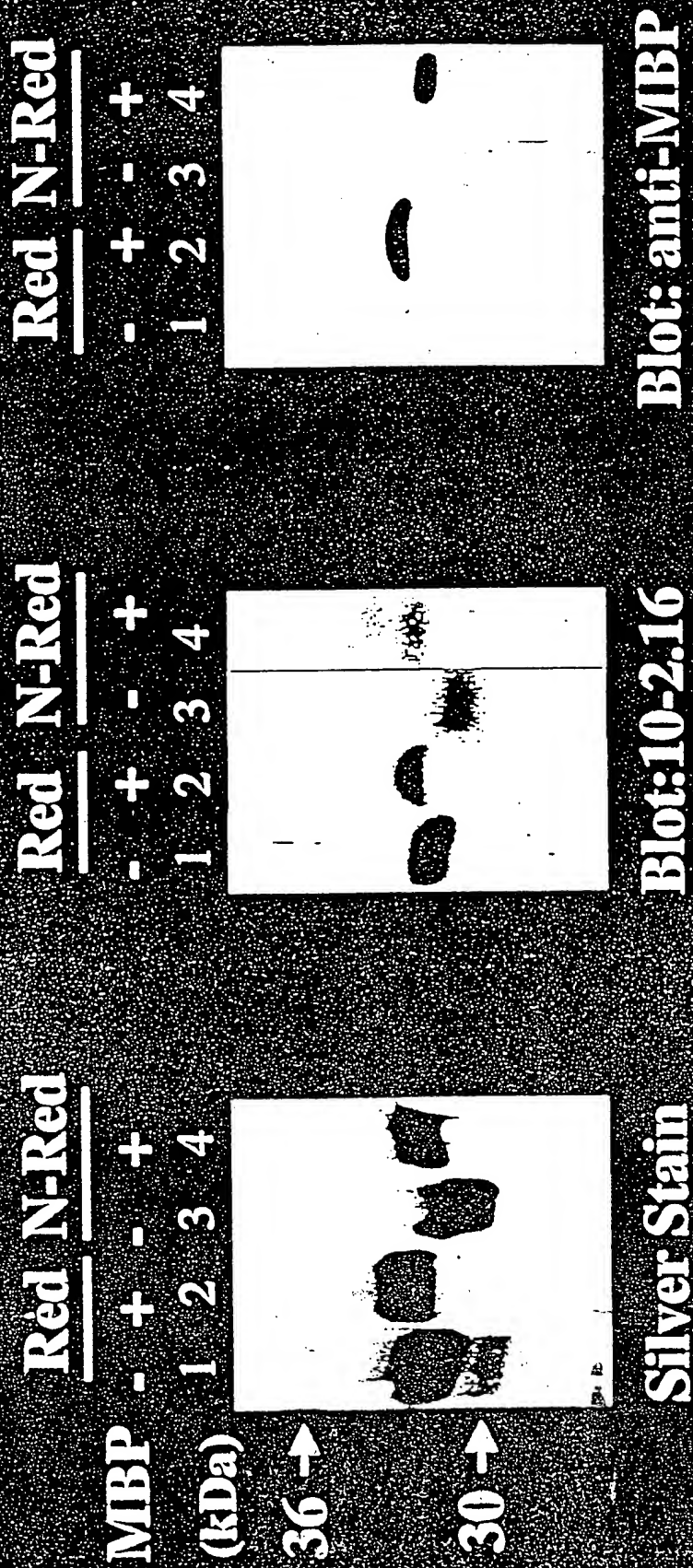


David H. Fremont, Wayne A. Hendrickson  
Philippa Marrack, John Kappler  
SCIENCE, 1996, 272:1001-1004

Figure 2



# Silver Staining and Western Analysis of Recombinant I-As.MBP. $\beta$ 1 $\alpha$ 1 Proteins





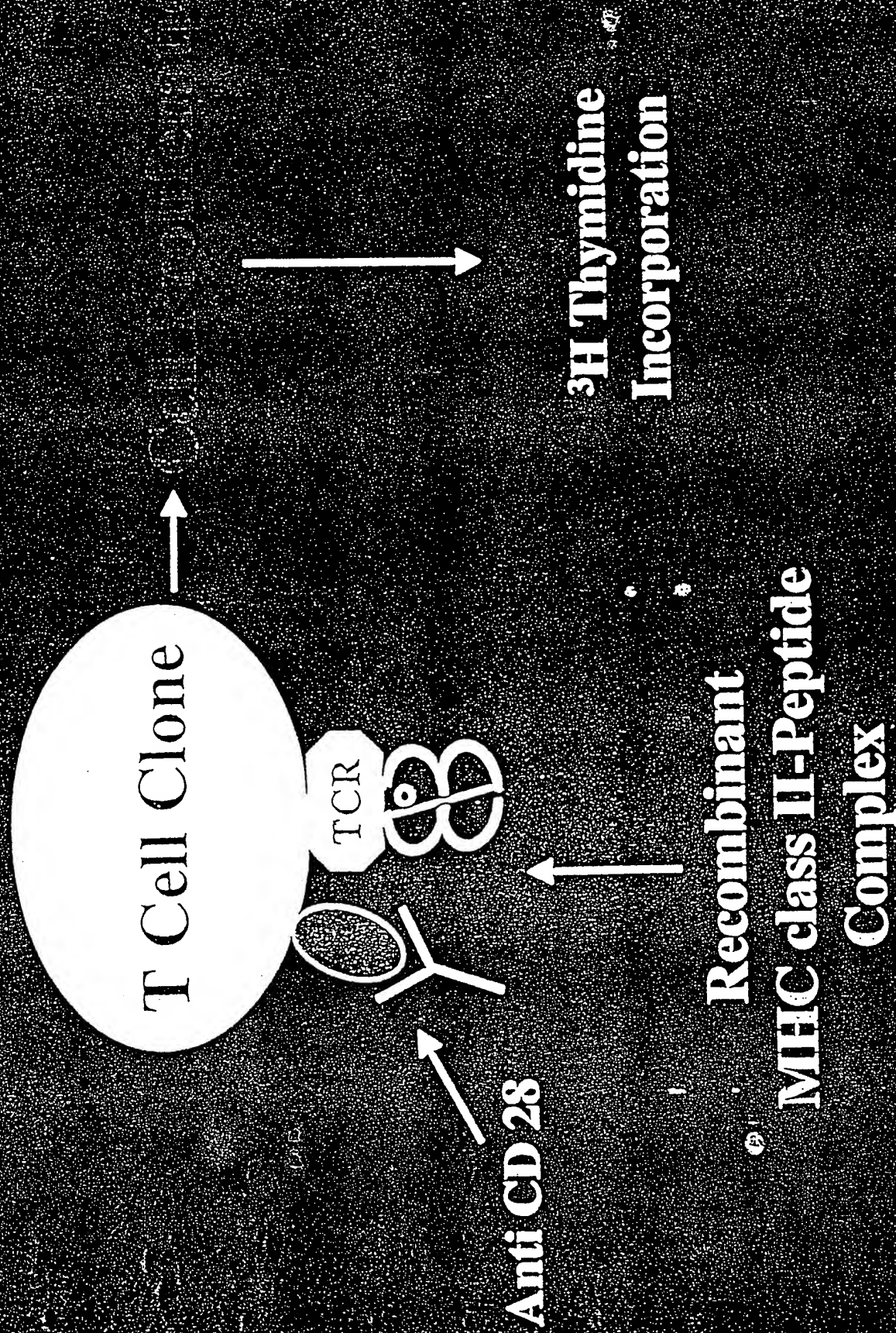
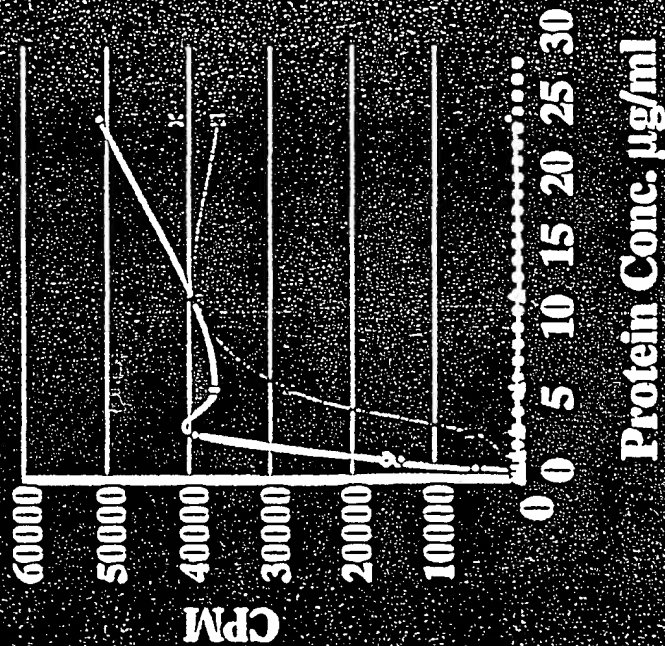


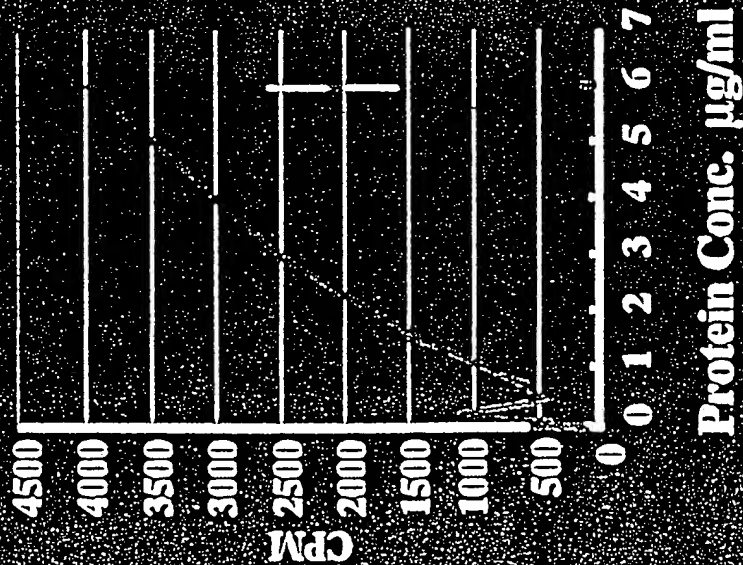
Figure 4

-- IAs.MBP.β1α1  
 APC+MBP(90-101)  
 -- IAs.β1α1  
 anti-CD3  
 APC alone  
 HS-1 alone



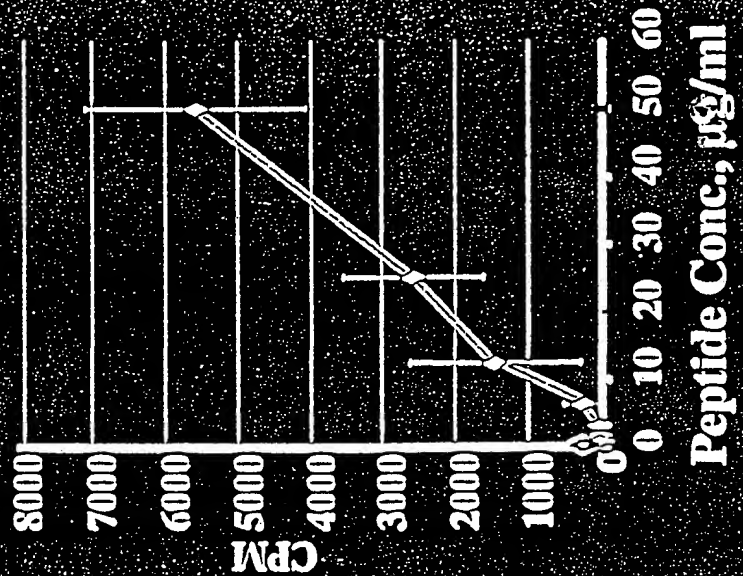
A

IAs.MBP.Ck  
 HS-1 alone  
 Anti-CD3



B

-- APC + Peptide  
 APC alone



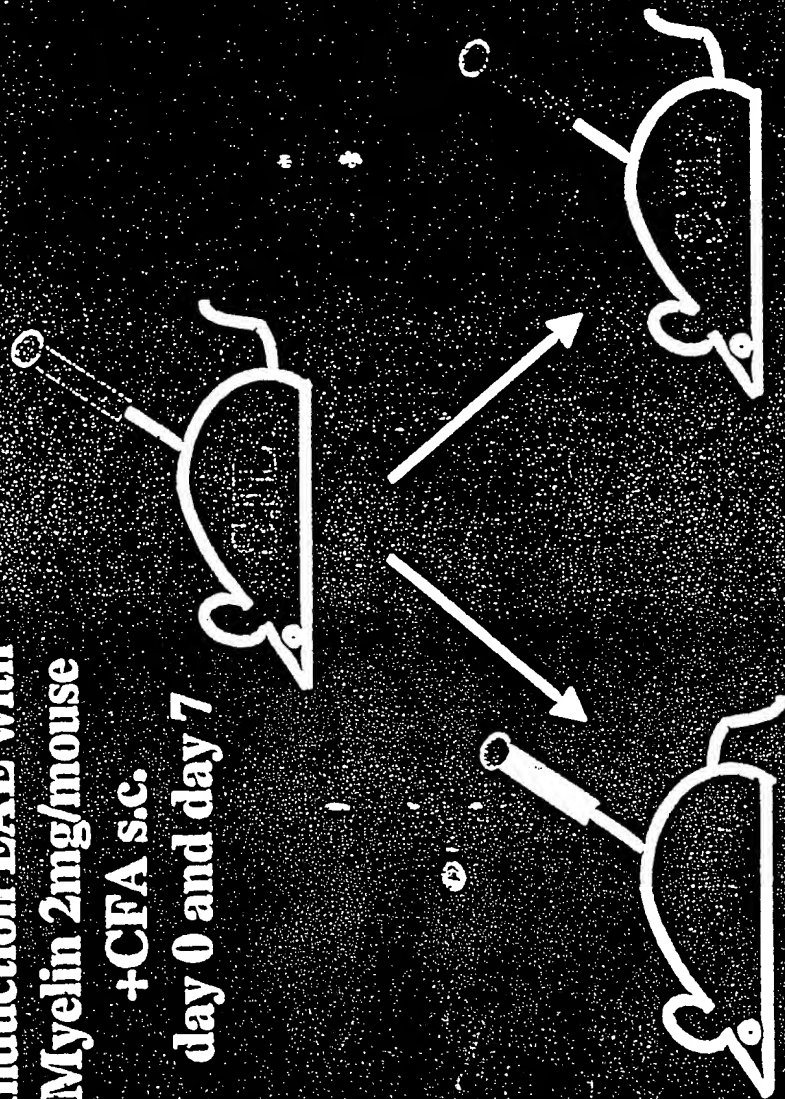
C

Figure 5

**Induction EAE with  
Myelin 2mg/mouse**

**+CFA s.c.**

**day 0 and day 7**



**Untreated  
none/PBS**

**Recombinant I-A<sup>s</sup>.MBP  
i.v. 10 µg/mouse**

**Inject at day 1, 4, 7, and 11  
Monitoring EAE at week 2-8**

## **EAE Score**

**0: Normal**

**0.5: Stiff tail**

**1.0: Limp tail**

**1.5: Limp tail with  
impaired righting reflex**

**2.0: Paralysis of one limb**

**2.5: Paralysis of one limb  
and weakness of the other**

**3.0: Complete paralysis  
of both hind limbs**

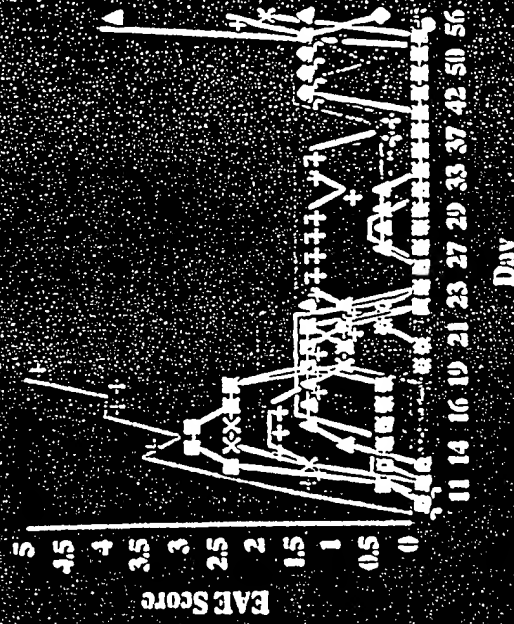
**4.0: Moribund**

**5.0: Death**



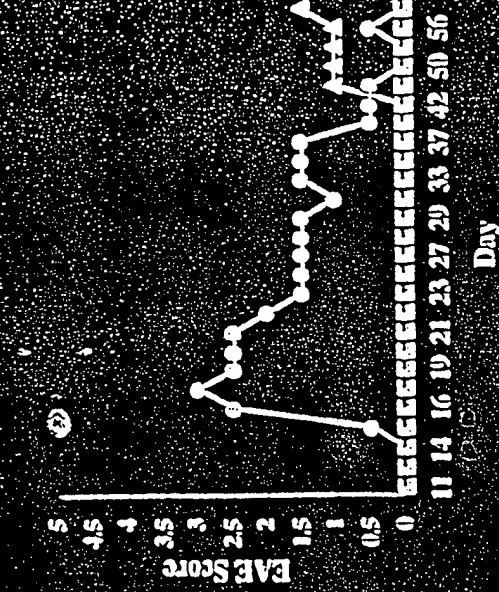
# A

Untreated



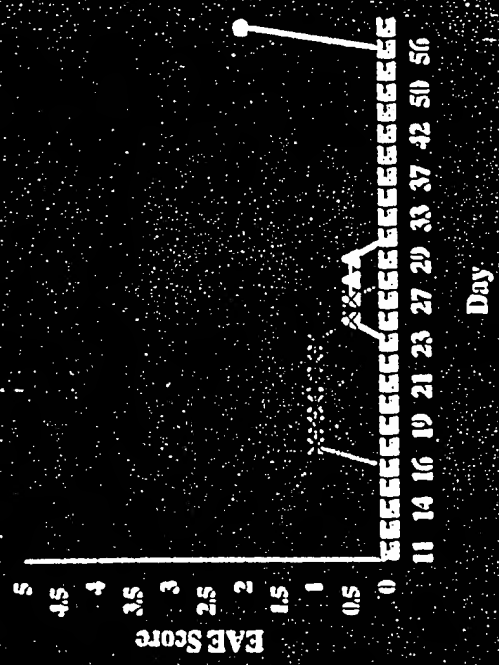
# B

I-ASMBP.Ck



# D

I-ASMBPβ1α1



# C

I-ASβ1α1

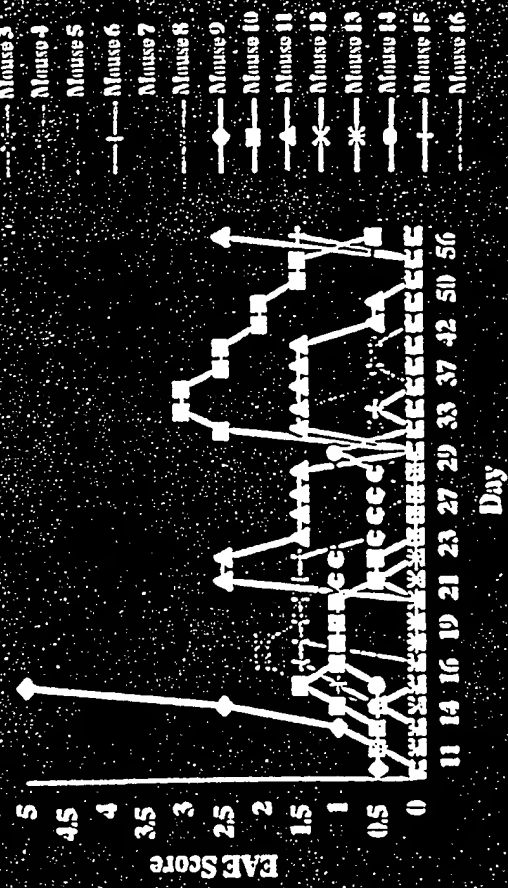
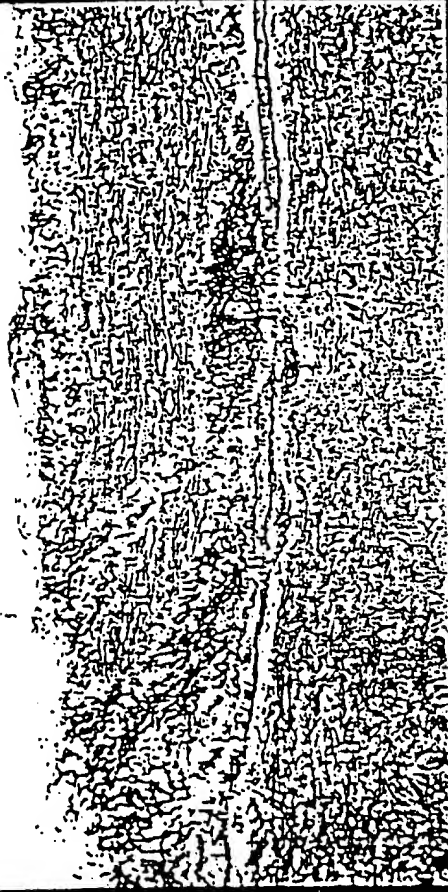
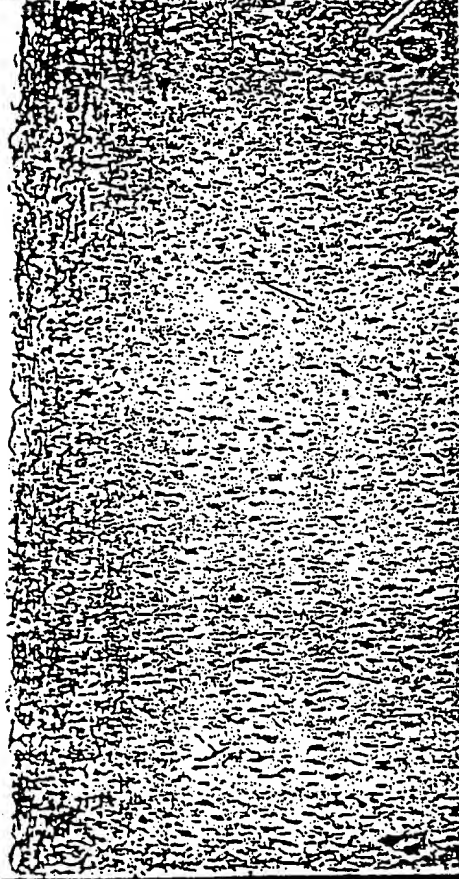


Figure 7

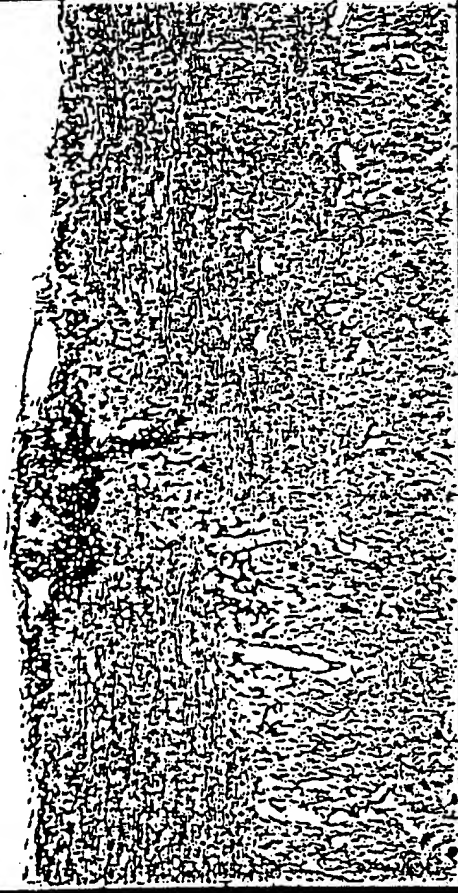
A. Untreated, histology score: 2.5



B. I-A<sup>S</sup>.MBP.Ck, histology score: 0



C. I-A<sup>S</sup>. $\beta$ 1 $\alpha$ 1, histology score: 2.0



D. I-A<sup>S</sup>.MBP. $\beta$ 1 $\alpha$ 1, histology score: 0.5

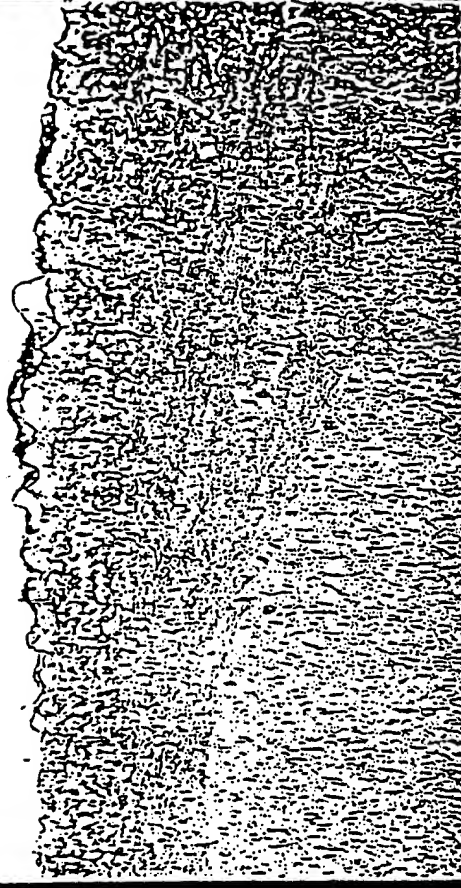
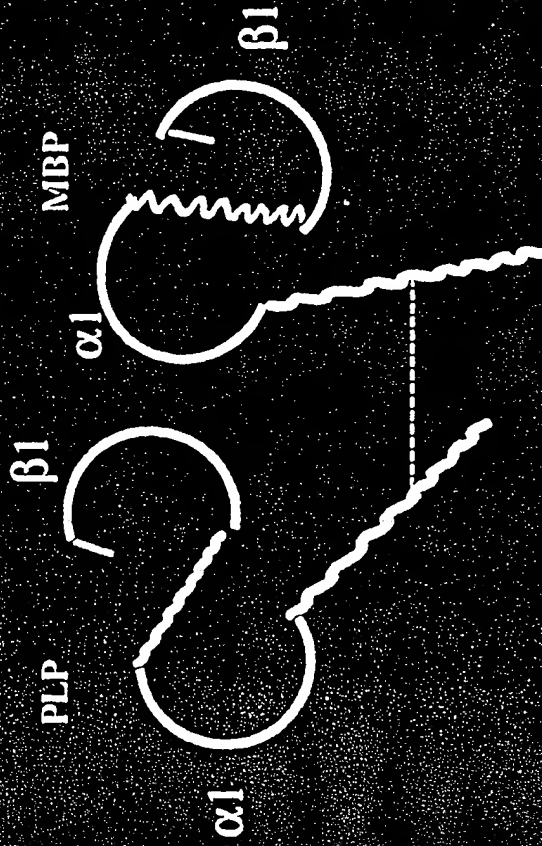


Figure 8

Schematic representation of heterodimeric truncated MHC class II-peptide molecules with two different peptide specificities.

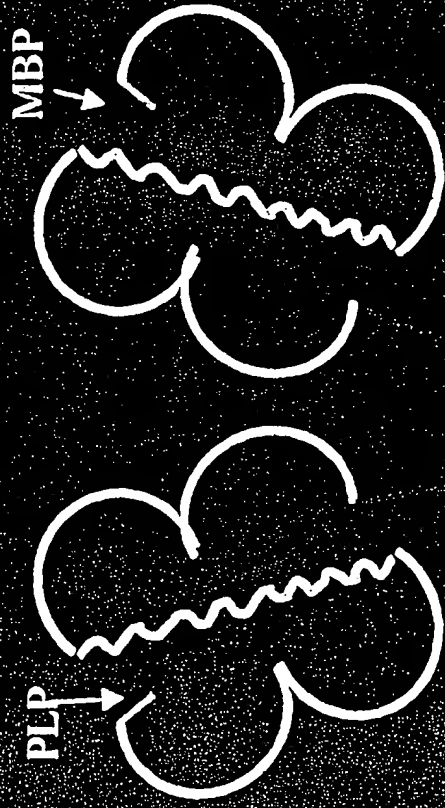


The dimerization can be achieved by the use site specific chemical conjugation or by the use of recombinant methods by the co-expression of MHC-Peptide molecules with different specificities having dimer forming amino acid tails (example: Ck, Leucine zipper)

Figure 9



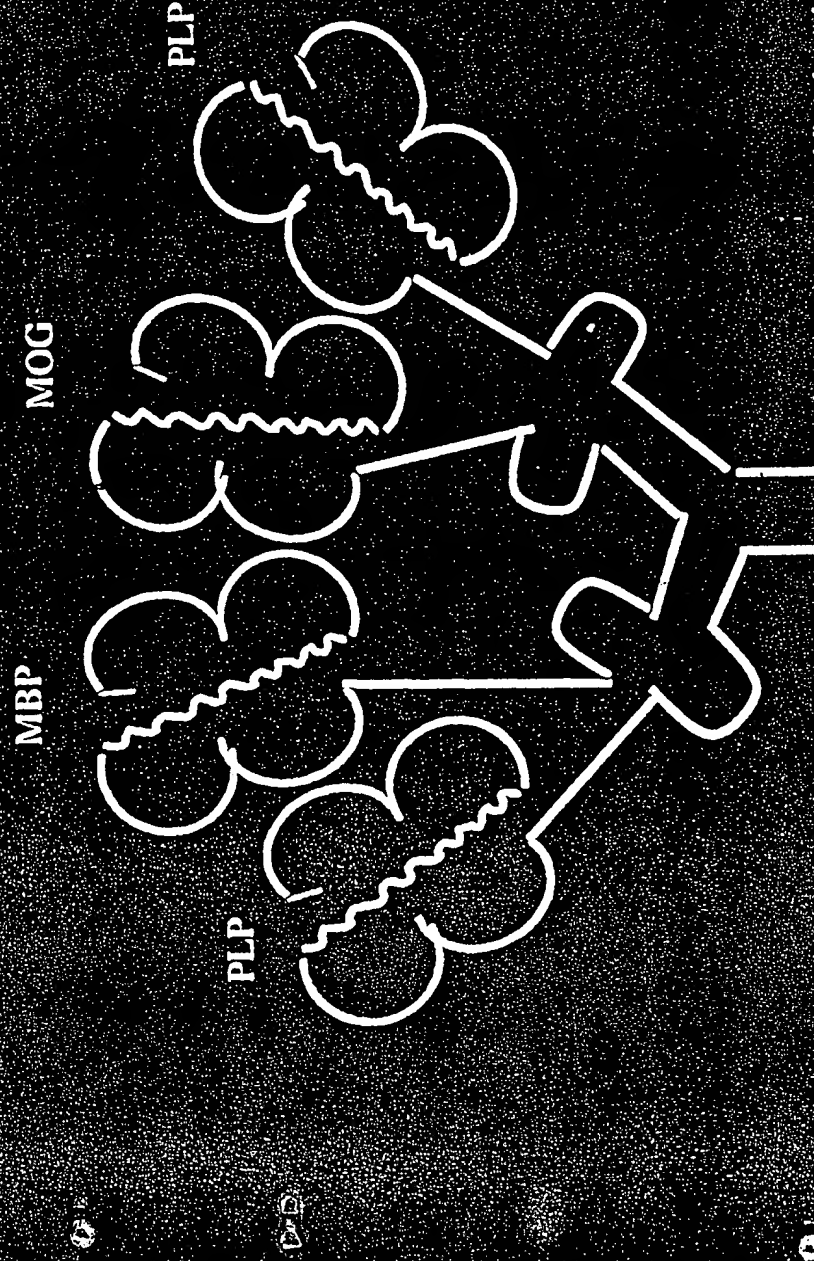
# Schematic representation of MHC-Peptide Heterodimer



The dimerization can be achieved by chemical conjugation of two different MHC-Peptide molecules or can be achieved by the use of recombinant methods by the co-expression MHC-Peptide molecules containing dimer-forming amino acid sequences (example: Lucine zipper, Ck. IgG heavy chain and light chain etc)

Figure 10

# Schematic representation of MHC-Peptide hetero-tetramer with binding capability to four different antigen specific T cells.

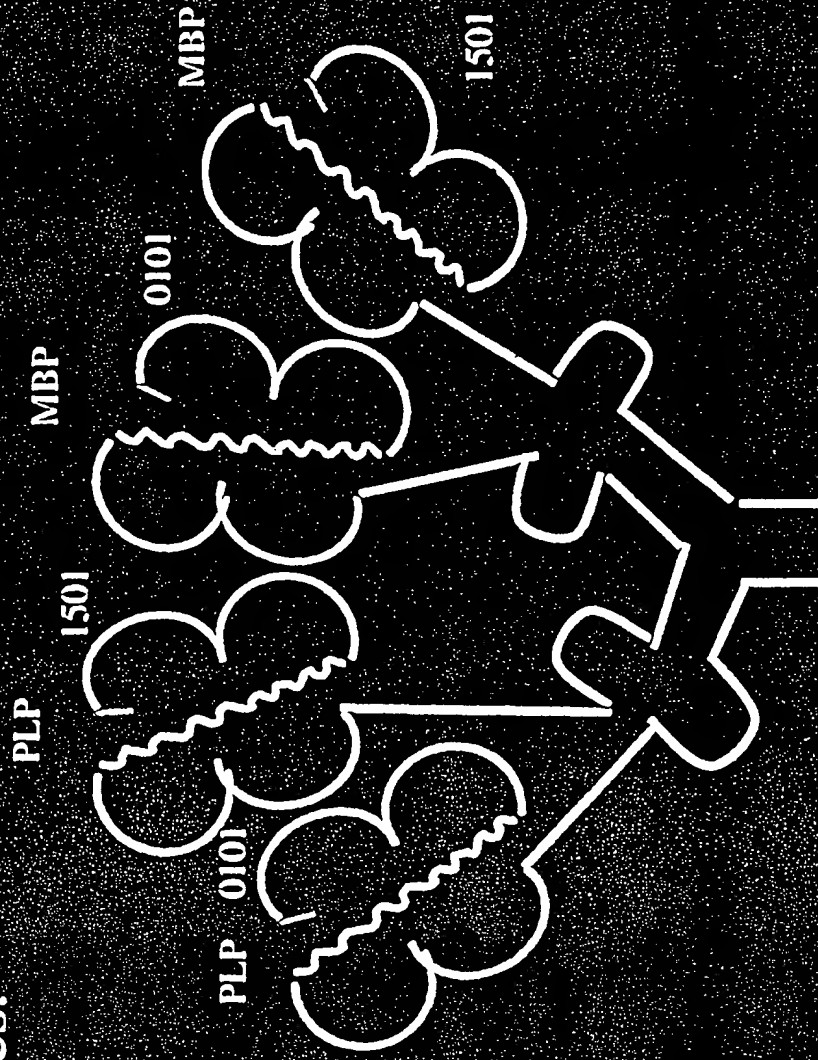


The tetramerization of MHC-peptide molecules can be achieved by site specific chemical conjugation of four different MHC peptide complexes or by recombinant methods. The recombinant methods include the appropriate use of IgG heavy and light chain tails to first express the heterodimer and then form the tetramer in vitro.

Figure 11



Schematic representation tetrameric MHC-Peptide complexes containing two different alleles of MHC class II and two different peptides.



DRB1\*1501 & DRB5\*0101 continuation  
with PLP & MBP & MOG peptides

Figure 12

Figure 1

# Effect of different recombinant MHC class II molecules on the development of EAE (day +60)

EAE induction Day 0 & 7	Treatment Day 1,4, 7 & 11 10 ug/injection i.v.	Incidence of disease (%)
Myelin 2 mg/mouse in CFA s.c.	none	12/16 (75%)
Myelin 2 mg/mouse in CFA s.c.	Monomer	2/12 (16 %)
Myelin 2 mg/mouse in CFA s.c.	Dimer	2/6 (33%)
Myelin 2 mg/mouse in CFA s.c.	Tetramer	3/14 (21%)
Myelin 2 mg/mouse in CFA s.c.	Monomer Pool	2/16 (12%)
Myelin 2 mg/mouse in CFA s.c.	Half Anergix	2/16 (12%)
Myelin 2 mg/mouse in CFA s.c.	I-As peptide	9/16 (56%)
Myelin 2 mg/mouse in CFA s.c.	Empty Half Anergix	10/16 (62%)

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